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## (54) NOVEL PROTEIN AND METHODS FOR THE PRODUCTION OF THE SAME

(57) A protein which inhibits osteoclast differentiation and/or maturation and a method of production of the protein. The protein is produced by human embryonic lung fibroblasts and has molecular weight of about 60 kD and about 120 kD under non-reducing conditions and about 60 kD under reducing conditions on SDS-polyacrylamide gel electrophoresis, respectively.

The protein can be isolated and purified from culture medium of the said fibroblasts. Furthermore, the protein can be produced by gene engineering.

The present invention includes cDNA for producing the protein by gene engineering, antibodies having specific affinity to the protein or a method for determination of the protein concentration using the antibodies.

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**Description****Field of the invention**

5 This invention relates to a novel protein, osteoclastogenesis inhibitory factor (OCIF), and methods for producing the protein.

**Background of the invention**

10 Human bones are always remodelling by the repeated process of resorption and reconstitution. In the process, osteoblasts and osteoclasts are considered to be the cells mainly responsible for bone formation and bone resorption, respectively. A typical example of disease caused by the progression of abnormal bone metabolism is osteoporosis. The disease is known to be provoked by the condition in which bone resorption by osteoclasts exceeds bone formation by osteoblasts, but the mechanism of osteoporosis has not yet been completely elucidated. Osteoporosis causes pain 15 in the bone and makes the bone fragile, leading to fracture. Since osteoporosis increases the number of bedridden old people, it has become a social issue with the increasing number of old people. Therefore, efficacious drugs for the treatment of the disease are expected to be developed. Bone mass reduction caused by the abnormal bone metabolism is thought to be prevented by inhibiting bone resorption, improving bone formation, or improving the balanced metabolism.

20 Bone formation is expected to be promoted by stimulating growth, differentiation, or activation of osteoblasts. Many cytokines are reported to stimulate growth or differentiation of osteoblasts, i.e. fibroblast growth factor (FGF) (Rodan S. B. et al., Endocrinology vol. 121, p1917, 1987), insulin-like growth factor-I (IGF-I) (Hock J.M. et al., Endocrinology vol. 122, p254, 1988), insulin-like growth factor-II (IGF-II) (McCarthy T. et al., Endocrinology vol. 124, p301, 1989), Activin A (Centrella M. et al., Mol. Cell. Biol. vol. 11, p250, 1991), Vasculotropin (Varonique M et al., Biochem. Biophys. Res. 25 Commun. vol. 199, p380, 1994), and bone morphogenetic protein (BMP) (Yamaguchi, A et al., J. Cell Biol. vol. 113, p682, 1991, Sampath T.K. et al., J. Biol Chem. vol. 267, p20532, 1992, and Knutson R. et al., Biochem. Biophys. Res. Commun. vol. 194, p1352, 1993).

30 On the other hand, cytokines which inhibits differentiation and/or maturation of osteoclasts have been paid attention and have been intensively studied. Transforming growth factor- $\beta$  (Chen C. et al., Proc. Natl. Acad. Sci. USA, vol. 85, p5683, 1988) and interleukin-4 (Kasano K. et al., Bone-Miner., vol. 21, p179, 1993) are found to inhibit the differentiation of osteoclasts. Calcitonin (Bone-Miner., vol. 17, p347, 1992), Macrophage colony-stimulating factor (Hattersley G. et al. J. Cell. Physiol. vol. 137, p199, 1988), interleukin-4 (Watanabe, K. et al., Biochem. Biophys. Res. Commun. vol. 172, p1035, 1990), and interferon- $\gamma$  (Gowen M. et al., J. Bone Miner. Res., vol. 1, p469, 1986) are found to inhibit bone resorption by osteoclasts.

35 These cytokines are expected to be efficacious drugs for improving bone mass reduction by stimulating bone formation and/or by inhibiting bone resorption. The cytokines such as insulin like growth factor-I and bone morphogenetic proteins are now investigated in clinical trials for their effects in treatment of patients with bone diseases. Calcitonin is already used as a drug to care osteoporosis and to diminish pain in osteoporosis.

40 Examples of drugs now clinically utilized for the treatment of bone diseases and for shortening the treatment period are dihydroxyvitamine D<sub>3</sub>, vitamin K<sub>2</sub>, calcitonin and its derivatives, hormones such as estradiol, ipriflavon, and calcium preparations. However, these drugs do not provide satisfactory therapeutic effects, and novel drug substances have been expected to be developed. As mentioned, bone metabolism is controlled in the balance between bone resorption and bone formation. Therefore, cytokines which inhibit osteoclast differentiation and/or maturation are expected to be developed as drugs for the treatment of bone diseases such as osteoporosis.

**45 Disclosure of Invention**

This invention was initiated from the view point described above. The purpose of this invention is to offer both a novel factor termed osteoclastogenesis inhibitory factor (OCIF) and a procedure to produce the factor efficiently.

50 The inventors have intensively searched for osteoclastogenesis inhibitory factors in human embryonic fibroblast IMR-90 (ATCC CCL186) conditioned medium and have found a novel osteoclastogenesis inhibitory factor (OCIF) which inhibits differentiation and/or maturation of osteoclasts.

The inventors have established a method for accumulating the protein to a high concentration by culturing IMR-90 cells using alumina ceramic pieces as the cell adherence matrices.

55 The inventors have also established an efficient method for isolating the protein, OCIF, from the IMR-90 conditioned medium using the following sequential column chromatography, ion-exchange, heparin affinity, cibacron-blue affinity, and reverse phase.

The inventors, based on the amino acid sequence of the purified natural OCIF, successfully cloned a cDNA encod-

ing this protein. The inventors established also a procedure to produce this protein which inhibits differentiation of osteoclasts. This invention concerns a protein which is produced by human lung fibroblast cells, has molecular weights in SDS-PAGE of 60 KD in the reducing conditions and 120 KD under the non-reducing conditions, has affinity for both cation-exchange resins and heparin, reduces its activity to inhibit differentiation and maturation of osteoclasts if treated for 5 10 minutes at 70 °C or for 30 minutes at 56 °C, and lose its activity to inhibit differentiation and maturation of osteoclasts by the treatment for 10 minutes at 90 °C. The amino acid sequence of the protein OCIF which is described in the present invention is clearly different from any of known factors inhibiting formation of osteoclasts.

The invention includes a method to purify OCIF protein, comprising ; (1) culturing human fibroblasts, (2) applying the conditioned medium to a heparin column to obtain the adsorbed fraction, (3) purifying the OCIF protein using a cation-exchange column, (4) purifying the OCIF protein using a heparin affinity column, (5) purifying the OCIF protein using a cibacron blue affinity column, (6) isolating the OCIF protein using reverse-phase column chromatography. Cibacron blue F3GA coupled to a carrier made of synthetic hydrophilic polymers is an example of materials used to prepare Cibacron blue columns. These columns are conventionally called "blue columns".

The invention includes a method for accumulating the OCIF protein to a high concentration by culturing human fibroblasts using alumina ceramic pieces as the cell-adherence matrices.

Moreover, the inventors determined the amino acid sequences of the peptides derived from OCIF, designed the primers based on these amino acid sequences, and obtained cDNA fragments encoding OCIF from a cDNA library of IMR-90 cells.

## 20 Detailed description of the invention

The OCIF protein of the present invention can be isolated from human fibroblast conditioned medium with high yield. The procedure to isolate OCIF is based on ordinary techniques for purifying proteins from biomaterials, in accordance with the physical and chemical properties of OCIF protein. For example, concentrating procedure includes ordinary biochemical techniques such as ultrafiltration, lyophylization, and dialysis. Purifying procedure includes combinations of several chromatographic techniques for purifying proteins such as ion-exchange column chromatography, affinity column chromatography, gel filtration column chromatography, hydrophobic column chromatography, reverse phase column chromatography, and preparative gel electrophoresis. The human fibroblast used for production of the OCIF protein is preferably IMR-90. A method for producing the IMR-90 conditioned medium is preferably a process comprising, adhering human embryonic fibroblast IMR-90 cells to alumina ceramic pieces in roller-bottles, using DMEM medium supplemented with 5 % new born calf serum for the cell culture, and cultivating the cells in roller-bottles for 7 to 10 days by stand cultivation. CHAPS (3-[(3-cholamid opropyl)-dimethylammonio]-1-propanesulfonate) is preferably added to the buffer as a detergent in the purification steps of OCIF protein.

OCIF protein of the instant invention can be initially obtained as a heparin binding basic OCIF fraction by applying the culture medium to a heparin column (Heparin-Sepharose CL-6B, Pharmacia), eluting with 10 mM Tris-HCl buffer, pH 7.5, containing 2 M NaCl, and then by applying the OCIF fraction to a Q + anion-exchange column (HiLoad-Q/FF, Pharmacia), and collecting non-adsorbed fraction. OCIF protein can be purified by subjecting the obtained OCIF fraction to purification on a S + cation-exchange column (HiLoad-S/FF, Pharmacia), a heparin column (Heparin-5PW, TOSOH), Cibacron Blue column (Blue-5PW, TOSOH), and a reverse-phase column (BU-300 C4, Perkin Elmer) and the material is defined by the previously described properties.

The present invention relates to a method of cloning cDNA encoding the OCIF protein based on the amino acid sequence of natural OCIF and a method of obtaining recombinant OCIF protein that inhibits differentiation and/or maturation of osteoclasts. The OCIF protein is purified according to the method described in the present invention and is treated with endopeptidase (for example, lysylendopeptidase). The amino acid sequences of the peptides produced by the digestion are determined and the mixture of oligonucleotides that can encode each internal amino acid sequence was synthesized. The OCIF cDNA fragment is obtained by PCR (preferably RT-PCR, reverse transcriptase PCR) using the oligonucleotide mixtures described above as primers. The full length OCIF cDNA encoding the OCIF protein is cloned from a cDNA library using the obtained OCIF DNA fragment as a probe. The OCIF cDNA containing the entire coding region is inserted into an expression vector. The recombinant OCIF can be produced by expressing the OCIF cDNA containing the entire coding region in mammalian cells or bacteria.

The present invention relates to the novel proteins OCIF2, OCIF3, OCIF4, and OCIF5 that are variants of OCIF and have the activity described above. These OCIF variants are obtained from the cDNA library constructed with IMR-90 poly(A) + RNA by hybridization using the OCIF cDNA fragment as a probe. Each of the OCIF variant cDNAs containing the entire coding region is inserted into an expression vector. Each recombinant OCIF variant can be produced by expressing each of the OCIF variant cDNAs containing the entire coding region in the conventional hosts. Each recombinant OCIF variant can be purified according to the method described in this invention. Each recombinant OCIF variant has an ability to inhibit osteoclastogenesis.

The present invention further includes OCIF mutants. They are substitution mutants comprising replacement of one

cysteine residue possibly involved in dimer formation with serine residue, and various deletion mutants of OCIF. Substitutions or deletions are introduced into the OCIF cDNA using polymerase chain reaction (PCR) or by restriction enzyme digestion. Each of these mutated OCIF cDNAs is inserted into a vector containing an appropriate promoter for gene expression. The resultant expression vector for each of the OCIF mutants is transfected into eukaryotic cells such as mammalian cells. Each of OCIF mutants can be obtained and purified from the conditioned media of the transfected cells.

5 The present invention provides polyclonal antibodies and a method to quantitatively determine OCIF concentration using these polyclonal antibodies.

As antigens (immunogens), natural OCIF obtained from IMR-90 conditioned medium, recombinant OCIF produced 10 by such hosts as microorganisms and eukaryotes using OCIF cDNA, synthetic peptides designed based on the amino acid sequence of OCIF, or peptides obtained from OCIF by partial digestion can be used. Anti-OCIF polyclonal antibodies are obtained by immunizing appropriate mammals with the antigens in combination with adjuvants for immunization if necessary, purifying from the serum by the ordinary purification methods. The anti-OCIF polyclonal antibodies which are labelled with radioisotopes or enzymes can be used in radio-immunoassay (RIA) system or immunoassay (EIA) 15 system. By using these assay systems, the concentrations of OCIF in biological materials such as blood and ascites and cells-culture medium can be easily determined.

The antibodies in the present invention can be used in radio immunoassay (RIA) or enzyme immunoassay (EIA). By using these assay systems, the concentration of OCIF in biological materials such as blood and ascites can be easily determined.

20 The present invention provides novel monoclonal antibodies and a method to quantitatively determine OCIF concentration using these monoclonal antibodies.

Anti-OCIF monoclonal antibodies can be produced by the conventional method using OCIF as an antigen. Native OCIF obtained from the culture medium of IMR-90 cells and recombinant OCIF produced by such hosts as microorganisms and eukaryotes using OCIF cDNA can be used as antigens. Alternatively, synthesized peptides designed based 25 on the amino acid sequence of OCIF and peptides obtained from OCIF by partial digestion can be also used as antigens. Immunized lymphocytes obtained by immunization of mammals with the antigen or by an in vitro immunization method were fused with myeloma of mammals to obtain hybridoma. The hybridoma clones secreting antibody which recognizes OCIF were selected from the hybridomas obtained by the cell fusion. The desired antibodies can be obtained by cell culture of the selected hybridoma clones. In preparation of hybridoma, small animals such as mice or 30 rats are generally used for immunization. To immunize, OCIF is suitably diluted with a saline solution (0.15 M NaCl), and is intravenously or intraperitoneally administered with an adjuvant to animals for 2-5 times every 2-20 days. The immunized animal was killed three days after final immunization, the spleen was taken out and the splenocytes were used as immunized B lymphocytes.

35 Mouse myeloma cell lines for cell fusion with the immunized B lymphocytes include, for example, p3/x63-Ag8, p3-U1, NS-1, MPC-11, SP-2/0, FO, p3x63 Ag8.653, and S194. Rat R-210 cell line may also be used. Human B lymphocytes are immunized by an in vitro immunization method and are fused with human myeloma cell line or EB virus transformed human B lymphocytes which are used as a parent cell line for cell fusion, to produce human type antibody.

40 Cell fusion of the immunized B lymphocytes and myeloma cell line is carried out principally by the conventional methods. For example, the method of Koehler G. et al. (Nature 256, 495-497, 1975) is generally used, and also an electric pulse method can be applied to cell fusion. The immunized B lymphocytes and transformed B cells are mixed at conventional ratios and a cell culture medium without FBS containing polyethylene glycol is generally used for cell fusion. The B lymphocytes fused with myeloma cell lines are cultured in HAT selection medium containing FBS to select hybridoma.

45 For screening of hybridoma producing anti-OCIF antibody, EIA, plaque assay, Ouchterlony, or agglutination assay can be principally used. Among them, EIA is simple and easy to operate with sufficient accuracy and is generally used. By EIA using purified OCIF, the desired antibody can be selected easily and accurately. Thus obtained hybridoma can be cultured by the conventional method of cell culture and frozen for stock if necessary. The antibody can be produced by culturing hybridoma using the ordinary cell culture method or by transplanting hybridoma intraperitoneally to animals. The antibody can be purified by the ordinary purification methods such as salt precipitation, gel filtration, and 50 affinity chromatography. The obtained antibody specifically reacts with OCIF and can be used for determination of OCIF concentration and for purification of OCIF. The antibodies of the present invention recognize epitopes of OCIF and have high affinity to OCIF. Therefore, they can be used for the construction of EIA. By (using) this assay system, the concentration of OCIF in biological materials such as blood and ascites can be easily determined.

55 The agents used for treating bone diseases that contain OCIF as an effective ingredient are provided by the present invention. Rats were subjected to denervation of left forelimb. Test compounds were administered daily after surgery for 14 days. After 2 weeks treatment, the animals were sacrificed and their forelimbs were dissected. Thereafter bones were tested for mechanical strength by three point bending method. OCIF improved mechanical strength of bone in a dose dependent manner.

The OCIF protein of the invention is useful as a pharmaceutical ingredients for treating or improving decreased bone mass in such as osteoporosis, bone diseases such as rheumatism, osteoarthritis, and abnormal bone metabolism in multiple myeloma. The OCIF protein is also useful as an antigen to establish immunological diagnosis of the diseases. Pharmaceutical preparations containing the OCIF protein as an active ingredients are formulated and can be orally or parenterally administered. The preparation contains the OCIF protein of the present invention as an efficacious ingredient and is safely administered to human and animals. Examples of the pharmaceutical preparations include compositions for injection or intravenous drip, suppositories, nasal preparations, sublingual preparations, and tapes for percutaneous absorption. The pharmaceutical preparation for injection can be prepared by mixing the pharmacologically efficacious amount of OCIF protein and pharmaceutically acceptable carriers. The carriers are vehicles and/or activators, e.g. amino acids, saccharides, cellulose derivatives, and other organic and inorganic compounds which are generally added to active ingredients. When the OCIF protein is mixed with the vehicles and/or activators to prepare injections, pH adjuster, buffer, stabilizer, solubilizing agent, etc. can be added, if necessary.

Brief description of the figures

15 Figure 1 shows the elution pattern of crude OCIF protein (Hiload-Q/FF pass-through fraction ; sample 3) from a Hiload-S/HP column.  
 Figure 2 shows the elution pattern of crude OCIF protein (heparin-5PW fraction ; sample 5) from a blue-5PW column.  
 20 Figure 3 shows the elution pattern of OCIF protein (blue-5PW fraction 49 to 50) from a reverse-phase column.  
 Figure 4 shows the SDS-PAGE of isolated OCIF proteins under reducing conditions or non-reducing conditions.

Description of the lanes,

25 lane 1,4 ; molecular weight marker proteins  
 lane 2,5 ; OCIF protein of peak 6 in figure 3  
 lane 3,6 ; OCIF protein of peak 7 in figure 3

Figure 5 shows the elution pattern of peptides obtained by the digestion of pyridyl ethylated OCIF protein digested with lysylendopeptidase, on a reverse-phase column.  
 30 Figure 6 shows the SDS-PAGE of isolated natural(n) OCIF protein and recombinant(r) OCIF proteins under non-reducing conditions. rOCIF(E) and rOCIF(C) were produced in 293/EBNA cells and in CHO cells, respectively.

Description of the lanes,

35 lane 1 ; molecular weight marker proteins  
 lane 2 ; a monomer type nOCIF protein  
 lane 3 ; a dimer type nOCIF protein  
 lane 4 ; a monomer type rOCIF(E) protein  
 lane 5 ; a dimer type rOCIF(E) protein  
 40 lane 6 ; a monomer type rOCIF(C) protein  
 lane 7 ; a dimer type rOCIF(C) protein

Figure 7 shows the SDS-PAGE of isolated natural(n) OCIF proteins and recombinant (r) OCIF proteins under reducing conditions. rOCIF(E) and rOCIF(C) were produced in 293/EBNA cells and in CHO cells, respectively.  
 Description of the lanes,

45 lane 8 ; molecular weight marker proteins  
 lane 9 ; a monomer type nOCIF protein  
 lane 10 ; a dimer type nOCIF protein  
 lane 11 ; a monomer type rOCIF(E) protein  
 50 lane 12 ; a dimer type rOCIF(E) protein  
 lane 13 ; a monomer type rOCIF(C) protein  
 lane 14 ; a dimer type rOCIF(C) protein

Figure 8 shows the SDS-PAGE of isolated natural(n) OCIF proteins and recombinant(r) OCIF proteins from which N-linked sugar chains were removed under reducing conditions. rOCIF(E) and rOCIF(C) are rOCIF protein produced in 293/EBNA cells and in CHO cells, respectively.

Description of the lanes,

lane 15 ; molecular weight marker proteins  
 lane 16 ; a monomer type nOCIF protein  
 lane 17 ; a dimer type nOCIF protein  
 lane 18 ; a monomer type rOCIF(E) protein  
 5 lane 19 ; a dimer type rOCIF(E) protein  
 lane 20 ; a monomer type rOCIF(C) protein  
 lane 21 ; a dimer type rOCIF(C) protein

Figure 9 shows comparison of amino acid sequences between OCIF and OCIF2.

10 Figure 10 shows comparison of amino acid sequences between OCIF and OCIF3.

Figure 11 shows comparison of amino acid sequences between OCIF and OCIF4.

Figure 12 shows comparison of amino acid sequences between OCIF and OCIF5.

Figure 13 shows standard curve for determination of OCIF protein concentration by an EIA employing anti-OCIF polyclonal antibodies.

15 Figure 14 shows standard curve for determination of OCIF protein concentration by an EIA employing anti-OCIF monoclonal antibodies.

Figure 15 shows the effect of rOCIF protein on osteoporosis.

#### Best Mode for Carrying Out the Invention

20 The present invention will be further explained by the following examples, however, the scope of the invention is not restricted to the examples.

#### EXAMPLE 1

##### Preparation of a conditioned medium of human fibroblast IMR-90

Human fetal lung fibroblast IMR-90 (ATCC-CCL186) cells were cultured on alumina ceramic pieces (80 g) (alumina: 99.5%, manufactured by Toshiba Ceramic K.K.) in DMEM medium (manufactured by Gibco BRL Co.) supplemented 30 with 5% CS and 10mM HEPES buffer (500 ml/roller bottle) at 37°C under the presence of 5% CO<sub>2</sub> for 7 to 10 days using 60 roller bottles (490 cm<sup>2</sup>, 110 x 171mm, manufactured by Coning Co.) in static culture. The conditioned medium was harvested, and a fresh medium was added to the roller bottles. About 30L of IMR-90 conditioned medium per batch culture was obtained. The conditioned medium was designated as sample 1.

#### 35 EXAMPLE 2

##### Assay method for osteoclast development inhibitory activity

Osteoclast development inhibitory activity was assayed by measuring tartrate-resistant acid phosphatase(TRAP) 40 activity according to the methods of M. Kumegawa et.al (Protein • Nucleic Acid • Enzyme, vol.34 p999, 1989) and N. Takahashi et.al (Endocrinology, vol.122, p1373, 1988 ) with modifications. Briefly, bone marrow cells obtained from 17 day-old mouse were suspended in  $\alpha$ -MEM (manufactured by GIBCO BRL Co.) containing 10% FBS,  $2 \times 10^{-8}$ M of activated vitamin D<sub>3</sub>, and each test sample, and were inoculated to each well of 96-well plate at a cell density of  $3 \times 10^5$  cells/0.2 ml/well. The plates were incubated for 7 days at 37°C in humidified 5%CO<sub>2</sub>. Cultures were further continued 45 by replacing 0.16 ml of old medium with the same volume of fresh medium on day 3 and day 5 after starting cultivation. On day 7, after washing the plates with phosphate buffered saline, cells were fixed with ethanol/acetone (1:1) for 1 min. at room temperature, and then osteoclast development was tested by determining for phosphatase activity using a kit (Acid Phosphatase, Leucocyte, Catalog No. 387-A, manufactured by Sigma Co.). The decrease of TRAP positive cells was taken as an indication of OCIF activity.

#### 50 EXAMPLE 3

##### Purification of OCIF

###### 55 i) Heparin Sepharose CL-6B column chromatography

The 90L of IMR-90 conditioned medium (sample 1) was filtrated with 0.22  $\mu$  membrane filter (hydrophilic Milidisk, 2000 cm<sup>2</sup>, Milipore Co.), and was divided into three portions. Each portion (30 l) was applied to a heparin Sepharose

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CL-6B column (5 x 4.1 cm, Pharmacia Co.) equilibrated with 10mM Tris-HCl containing 0.3M NaCl, pH 7.5. After washing the column with 10mM Tris-HCl, pH 7.5 at a flow rate of 500 ml/hr., heparin Sepharose CL-6B adsorbent protein fraction was eluted with 10mM Tris-HCl, pH 7.5, containing 2M NaCl. The fraction was designated as sample 2.

5      ii) HiLoad-Q/FF column chromatography

The heparin Sepharose-adsorbent fraction (sample 2) was dialyzed against 10mM Tris-HCl, pH 7.5, supplemented with CHAPS to a final concentration of 0.1%, incubated at 4 °C overnight, and divided into two portions. Each portion was then applied to an anion-exchange column (HiLoad-Q/FF, 2.6 x 10 cm, Pharmacia Co.) which was equilibrated with 10mM Tris-HCl, 0.1% CHAPS, pH 7.5 to obtain a non-adsorbent fraction (1000 ml). The fraction was designated as sample 3.

iii) HiLoad-S/HP column chromatography

15     The HiLoad-Q non-adsorbent fraction (sample 3) was applied to a cation-exchange column (HiLoad-S/HP, 2.6 x 10 cm, Pharmacia Co.) which was equilibrated with 50 mM Tris-HCl, 0.1% CHAPS, pH 7.5. After washing the column with 50 mM Tris-HCl, 0.1% CHAPS, pH 7.5, the adsorbed protein was eluted with linear gradient from 0 to 1 M NaCl at a flow rate of 8 ml/min for 100 min. and fractions (12 ml) were collected. Each ten fractions from number 1 to 40 was pooled to form one portion. Each 100 µl of the four portions was tested for OCIF activity. OCIF activity was observed in 20 fractions from 11 to 30 (as shown in Figure 1). The fractions from 21 to 30 which had higher specific activity were collected and was designated as sample 4.

iv) Heparin-5PW affinity column chromatography

25     One hundred and twenty ml of HiLoad-S fraction from 21 to 30 (sample 4) was diluted with 240 ml of 50 mM Tris-HCl, 0.1% CHAPS, pH 7.5, and applied to heparin-5PW affinity column (0.8 x 7.5 cm, Tosoh Co.) which was equilibrated with 50mM Tris-HCl, 0.1% CHAPS, pH 7.5. After washing the column with 50mM Tris-HCl, 0.1% CHAPS, pH 7.5, the adsorbed protein was eluted with linear gradient from 0 to 2M NaCl at a flow rate of 0.5ml/min for 60 min. and fractions (0.5 ml) were collected. Fifty µl was removed from each fraction to test for OCIF activity. The active fractions, 30     eluted with 0.7 to 1.3M NaCl was pooled and was designated as sample 5.

v) Blue 5PW affinity column chromatography

35     Ten ml of sample 5 was diluted with 190 ml of 50mM Tris-HCl, 0.1% CHAPS, pH 7.5 and applied to a blue-5PW affinity column, (0.5x5 cm, Tosoh Co.) which was equilibrated with 50mM Tris-HCl, 0.1% CHAPS, pH 7.5. After washing the column with 50mM Tris-HCl, 0.1% CHAPS, pH7.5, the adsorbed protein was eluted with a 30 ml linear gradient from 0 to 2M NaCl at a flow rate of 0.5 ml/min., and fractions (0.5 ml) were collected. Using 25 µl of each fraction, OCIF activity was evaluated. The fractions number 49 to 70, eluted with 1.0-1.6M NaCl had OCIF activity.

40     vi) Reverse phase column chromatography

The blue 5PW fraction obtained by collecting fractions from 49 to 50 was acidified with 10µl of 25% TFA and applied to a reverse phase C4 column (BU-300, 2.1x220mm, manufactured by Perkin-Elmer) which was equilibrated with 0.1% of TFA and 25% of acetonitrile. The adsorbed protein was eluted with linear gradient from 25 to 55% acetonitrile at a 45     flow rate of 0.2 ml/min. for 60 min., and each protein peak was collected (Fig.3). One hundred µl of each peak fraction was tested for OCIF activity, and peak 6 and the peak 7 had OCIF activity. The result was shown in Table 1.

Table 1

OCIF activity eluted from reverse phase C4 column				
Sample	Dilution			
	1/40	1/120	1/360	1/1080
Peak 6	++	++	+	-
Peak 7	++	+	-	-

[ ++ means OCIF activity inhibiting osteoclast development more than 80%, + means OCIF activity inhibiting osteoclast development between 30% and 80%, and - means no OCIF activity.]

15

## EXAMPLE 4

## Molecular weight of OCIF protein

20 The two protein peaks (6 and 7) with OCIF activity were subjected to SDS-polyacrylamide gel electrophoresis under reducing and non-reducing conditions. Briefly, 20 $\mu$ l of each peak fraction was concentrated under vacuum and dissolved in 1.5 $\mu$ l of 10mM Tris-HCl, pH 8, 1mM EDTA, 2.5% SDS, 0.01% bromophenol blue, and incubated at 37°C overnight under non-reducing conditions or under reducing conditions (with 5% of 2-mercaptoethanol). Each 1.0  $\mu$ l of sample was then analyzed by SDS-polyacrylamide gel electrophoresis with a gradient gel of 10-15% acrylamide (Pharmacia Co.) and an electrophoresis-device (Fast System, Pharmacia Co.). The following molecular weight marker proteins were used to calculate molecular weight : phosphorylase b (94 kD), bovine serum albumin (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), trypsin inhibitor (20.0 kD), and lactalbumin (14.4 kD). After electrophoresis, protein bands were visualized by silver stain using Phast Silver Stain Kit. The results were shown in Fig. 4.

25 A protein band with an apparent 60 KD was detected in the peak 6 protein under both reducing and non-reducing conditions. A protein band with an apparent 60 KD was detected under reducing conditions and a protein band with an apparent 120 KD was detected under non-reducing conditions in the peak 7 protein. Therefore, the protein of peak 7 was considered to be a homodimer of the protein of peak 6.

## EXAMPLE 5

35

## Thermostability of OCIF

30 Twenty  $\mu$ l of sample from the blue-5PW fractions 51 and 52 was diluted to 30 $\mu$ l with 10 mM phosphate buffered saline, pH 7.2, and incubated for 10 min. at 70°C or 90 °C, or for 30 min. at 56°C. The heat-treated samples were tested 40 for OCIF activity. The results were shown in Table 2.

Table 2

Thermostability of OCIF			
Sample	Dilution		
	1/300	1/900	1/2700
untreated	++	+	-
70°C, 10 min	+	-	-
56°C, 30 min	+	-	-
90°C, 10 min	-	-	-

[ ++ means OCIF activity inhibiting osteoclast development more than 80%, + means OCIF activity inhibiting osteoclast development between 30% and 80%, and - means no OCIF activity.]

## EXAMPLE 6

## Internal amino acid sequence of OCIF protein

5 Each 2 fractions (1 ml) from No. 51-70 of blue-5PW fraction was acidified with 10  $\mu$ l of 25% TFA, and was applied to a reverse phase C4 column (BU-300, 2.1x220mm, manufactured by Perkin-Elmer Co.) equilibrated with 25% of acetonitrile containing 0.1 % TFA. The adsorbed protein was eluted with a 12 ml linear gradient of 25 to 55% acetonitrile at a flow rate of 0.2 ml/min, and the protein fractions corresponding to peak 6 and peak 7 were collected, respectively. The protein of each peak was applied to a protein sequencer (PROCISE 494, Perkin-Elmer Co.). However, the N-terminal sequence of the protein of each peak could not be analyzed. Therefore, N-terminal of the protein of each peak was considered to be blocked. So, internal amino acid sequences of these proteins were analyzed.

10 The protein of peak 6 or peak 7 purified by C4-HPLC was concentrated by centrifugation and pyridylethylated under reducing conditions. Briefly, 50  $\mu$ l of 0.5 M Tris-HCl, pH 8.5, containing 100 $\mu$ g of dithiothreitol, 10mM EDTA, 7 M guanidine-HCl, and 1% CHAPS was added to each samples, and the mixture was incubated overnight in the dark at a room 15 temperature. Each the mixture was acidified with 25% TFA (a final concentration 0.1%) and was applied to a reversed phase C4 column (BU-300, 2.1x30mm, Perkin-Elmer Co.) equilibrated with 20 % acetonitrile containing 0.1 % TFA. The pyridil-ethylated OCIF protein was eluted with a 9 ml linear gradient from 20 to 50% acetonitrile at a flow rate of 0.3 ml/min, and each protein peak was collected. The pyridil-ethylated OCIF protein was concentrated under vacuum , and dissolved in 25 $\mu$ l of 0.1 M Tris-HCl, pH 9, containing 8 M Urea, and 0.1 % Tween 80. Seventy three  $\mu$ l of 0.1 M Tris-HCl, 20 pH 9, and 0.02  $\mu$ g of lysyl endopeptidase (Wako Pure Chemical, Japan) were added to the tube, and incubated at 37 °C for 15 hours. Each digest was acidified with 1  $\mu$ l of 25% TFA and was applied to a reverse phase C8 column (RP-300, 2.1x220mm, Perkin-Elmer Co.) equilibrated with 0.1% TFA.

25 The peptide fragments were eluted from the column with linear gradient from 0 to 50 % acetonitrile at a flow rate of 0.2 ml/min for 70 min., and each peptide peak was collected. Each peptide fragment (P1 - P3) was applied to the protein sequencer. The sequences of the peptides were shown in Sequence Numbers 1 - 3, respectively.

## EXAMPLE 7

## Determination of nucleotide sequence of the OCIF cDNA

30 i) Isolation of poly(A) + RNA from IMR-90 cells

35 About 10 ug of poly(A) + RNA was isolated from  $1 \times 10^8$  cells of IMR-90 by using Fast Track mRNA isolation kit (Invitrogen) according to the manufacturer's instructions.

ii) Preparation of mixed primers

40 The following two mixed primers were synthesized based on the amino acid sequences of two peptides (peptide P2 and peptide P3, sequence numbers 2 and 3, respectively). All the oligonucleotides in the mixed primers No. 2F can code for the amino acid sequence from the sixth residue, glutamine (Gln) to the twelfth residue, leucine (Leu), in peptide P2. All the oligonucleotides in the mixed primers No. 3R can code for the amino acid sequence from the sixth residue, histidine (His), to the twelfth residue, lysine (Lys), in peptide P3. The sequences of the mixed primers No. 2F and No. 3R were shown in Table 3.

45

50

55

Table 3

5

No. 2F

10

5' -CAAGAACAAA CTTTCAATT-3'

G G G C C GC

A

15

G

20

No. 3R

5' -TTTATACATT GTAAAAGAAT G-3'

C G C G GCTG  
A C  
G T

30

35 iii) Amplification of OCIF cDNA fragment by PCR (Polymerase chain reaction)

First strand cDNA was generated using Superscript II cDNA synthesis kit (Gibco BRL) and 1 ug of poly (A) + RNA obtained in the example 7-i) according to the manufacturer's instructions. The DNA fragment encoding OCIF was obtained by PCR using the cDNA template and the primers shown in EXAMPLE 7-ii).

40 PCR was performed with the conditions as follows;

45

10X Ex Taq Buffer (Takara Shuzo)	5 ul
2.5 mM solution of dNTPs	4 ul
cDNA solution	1 ul
Ex Taq (Takara Shuzo)	0.25 ul
sterile distilled water	29.75 ul
40 uM solution of primers No. 2F	5 ul
40 uM solution of primers No. 3R	5 ul

50

55 The components of the reaction were mixed in a microcentrifuge tube. An initial denaturation step at 95 °C for 3 min was followed by 30 cycles of denaturation at 95°C for 30 sec annealing at 50 °C for 30 sec and extention at 70 °C for 2min. After the amplification, final extention step was performed at 70 °C for 5min. The size of PCR products were determined on a 1.5 % agarose gel electrophoresis. About 400 bp OCIF DNA fragment was obtained.

## EXAMPLE 8

Cloning of the OCIF cDNA fragment amplified by PCR and determination of its DNA sequence

5 The OCIF cDNA fragment amplified by PCR in EXAMPLE 7-iii) was inserted in the plasmid, pBluescript II SK<sup>+</sup> using  
 DNA ligation kit ver. 2 (Takara Shuzo) according to the method by Marchuk, D. et al. (Nucleic Acids Res., vol 19, p1154,  
 1991). E.coli. DH5  $\alpha$  (Gibco BRL) was transformed with ligation mixture. The transformants were grown and a plasmid  
 containing the OCIF cDNA (about 400 bp) was purified using the commonly used method. This plasmid was called  
 pBSOCIF. The sequence of OCIF cDNA in pBSOCIF was determined using Taq Dye Deoxy Terminator Cycle Sequenc-  
 ing kit (Perkin Elmer). The size of the OCIF cDNA is 397 bp. The OCIF cDNA encodes an amino acid sequence con-  
 taining 132 residues. The amino acid sequences of the internal peptides (peptide P2 and peptide P3, sequence number  
 10 2 and 3, respectively) that were used to design the primers were found at N- or C-terminal side in the amino acid  
 sequence of the 132 amino acid polypeptide predicted by the 397 bp OCIF cDNA. In addition, the amino acid sequence  
 of the internal peptide P1 (sequence number 1) was also found in the predicted amino acid sequence of the polypep-  
 15 tide. These data show that the 397 bp OCIF cDNA is a portion of the full length OCIF cDNA.

## EXAMPLE 9

Preparation of the DNA probe

20 The 397 bp OCIF cDNA was prepared according to the conditions described in EXAMPLE 7-iii). The OCIF cDNA  
 was subjected to a preparative agarose gel electrophoresis. The OCIF cDNA was purified from the gel using QIAEX gel  
 extraction kit (QIAGEN), labeled with [ $\alpha$ <sup>32</sup>P]dCTP using Megaprime DNA labeling system (Amersham) and used to  
 select a phage containing the full length OCIF cDNA.

## EXAMPLE 10

Preparation of the cDNA library

30 cDNA was generated using Great Lengths cDNA synthesis kit (Clontech), oligo (dT) primer, [ $\alpha$ <sup>32</sup>P]dCTP and 2.5  
 ug of poly(A) + RNA obtained in the example 7-i) according to the manufacturer's instructions. EcoRI-Sall-NotI adaptor  
 was ligated to the cDNA. The cDNA was separated from the free adaptor and unincorporated free [ $\alpha$ <sup>32</sup>P]dCTP. The  
 purified cDNA was precipitated with ethanol and dissolved in 10 ul of TE buffer (10 mMTris-HCl (pH8.0), 1 mM EDTA).  
 The cDNA with the adaptor was inserted in  $\lambda$ ZAP EXPRESS vector (Stratagene) at EcoRI site. The recombinant  $\lambda$ ZAP  
 35 EXPRESS phage DNA containing the cDNA was in vitro packaged using Gigapack gold II packaging extract (Strata-  
 gene) and recombinant  $\lambda$ ZAP EXPRESS phage library was prepared.

## EXAMPLE 11

40 Screening of recombinant phage

Recombinant phages obtained in EXAMPLE 10 were infected to E. Coli, XL1-Blue MRF' (Stratagene) at 37 °C for  
 15 min.. The infected E.coli cells were added to NZY medium containing 0.7 % agar at 50°C and plated on the NZY  
 45 agar plates. After the plates were incubated at 37 °C overnight, Hybond N (Amersham) were placed on the surface of  
 plates containing plaques. The membranes were denatured in the alkali solution, neutralized, and washed in 2xSSC  
 according to the standard protocol. The phage DNA was immobilized on the membranes using UV Crosslink (Strata-  
 gene). The membranes were incubated in the hybridization buffer (Amersham) containing 100  $\mu$ g/ml salmon sperm  
 DNA at 65°C for 4 hours and then incubated at 65 °C overnight in the same buffer containing 2x10<sup>5</sup> cpm/ml denatured  
 50 OCIF DNA probe. The membranes were washed twice with 2xSSC and twice with a solution containing 0.1xSSC and  
 0.1 % SDS at 65 °C for 10 min each time. The positive clones were purified by repeating the screening twice. The puri-  
 fied  $\lambda$ ZAP EXPRESS phage clone containing about 1.6 kb DNA insert was used in the experiments described below.  
 This phage was called  $\lambda$ OCIF. The purified  $\lambda$ OCIF and the infected into E. Coli XL1-Blue MRF' (Stratagene) according  
 to a protocol of  $\lambda$ ZAP EXPRESS cloning kit (Stratagene). The culture broth of infected XL1-Blue MRF' was prepared.  
 Purified 1OCIF and ExAssist helper phage (Stratagene) were co-infected into E. coli strain XL-1 blue MRF' according  
 55 to the protocol supplied with the kit. The culture broth of the co-infected XL-1 blue MRF' was added to a culture of E.  
 coli strain XLOR (Stratagene) to transform them. Thus we obtained a Kanamycin-resistant transformant harboring a  
 plasmid designated pBKOCIF which is a pBKCMV (Stratagene) vector containing the 1.6 kb insert fragment.  
 The transformant including the plasmid containing about 1.6 kb OCIF cDNA was obtained by picking up the kanamycin-

resistant colonies. The plasmid was called pBKOCIF. The transformant has been deposited to National Institute of Bio-science and Human-Technology (NIBH), Agency of Industrial Science and Technology as "FERM BP-5267" as pBK/O1F10. A national deposit (Accession number, FERM P-14998) was transferred to the international deposit, on October 25, 1995 according to the Budapest treaty. The transformant pBK/O1F10 was grown and the plasmid pBKOCIF 5 was purified according to the standard protocol.

## EXAMPLE 12

Determination of the nucleotide sequence of OCIF cDNA containing the full coding region.

10 The nucleotide sequence of OCIF cDNA obtained in EXAMPLE 11 was determined using Taq Dye Deoxy Terminator Cycle Sequencing kit (Perkin Elmer). The primers used were T3, T7 primers (Stratagene) and synthetic primers designed according to the OCIF cDNA sequence. The sequences of these primers are shown in sequence numbers 16 to 29. The nucleotide sequence of the OCIF cDNA is shown in sequence number 6 and the amino acid sequence predicted by the cDNA sequence is shown in sequence number 5.

## EXAMPLE 13

20 Production of recombinant OCIF by 293/EBNA cells

## i) Construction of the plasmid for expressing OCIF cDNA

25 pBKOCIF containing about 1.6 kb OCIF cDNA was prepared as described in EXAMPLE 11, and digested with restriction enzymes, BamHI and Xhol. The OCIF cDNA insert was cut out, separated by an agarose gel electrophoresis, and purified using QIAEX gel extraction kit (QIAGEN). The purified OCIF cDNA insert was ligated using DNA ligation kit ver. 2 (Takara Shuzo) to the expression vector pCEP4 (Invitrogen) digested with restriction enzymes, BamHI and Xhol. E.coli. DH5 $\alpha$  (Gibco BRL) was transformed with the ligation mixture. The transformants were grown and the plasmid containing the OCIF cDNA (about 1.6 kb) was purified using QIAGEN column (QIAGEN). The expression plasmid pCEPOCIF was precipitated with ethanol, and dissolved in sterile distilled water was used in the expriments 30 described below.

## ii) Transient expression of OCIF cDNA and analysis of the biological activity

35 Recombinant OCIF was produced using the expression plasmid, pCEPOCIF prepared in EXAMPLE 13-i) according to the method described below.  $8 \times 10^5$  cells of 293/EBNA (Invitrogen) were inoculated in each well of the 6-well plate using IMDM containing 10 % fetal calf serum (Gibco BRL). After the cells were incubated for 24 hours, the culture medium was removed and the cells were washed with serum free IMDM. The expression plasmid, pCEPOCIF and lipofectamine (Gibco BRL) were diluted with OPTI-MEM (Gibco BRL) and were mixed, and added to the cells in each well 40 according to the manufacture's instructions. Three  $\mu$ g of pCEPOCIF and 12  $\mu$ l of lipofectamine were used for each transfection. After the cells were incubated with pCEPOCIF and lipofectamine for 38 hours, the medium was replaced with 1 ml of OPTI-MEM. After the transfected cells were incubated for 30 hours, the conditioned medium was harvested and used for the biological assay. The biological activity of OCIF was analysed according to the method described 45 below. Bone marrow cells obtained from mice, 17 days-old, were suspended in  $\alpha$ -MEM (manufactured by GIBCO BRL Co.) containing 10% FBS,  $2 \times 10^{-8}$ M activated vitamin D<sub>3</sub>, and each test sample, and were inoculatd and cultured for 7 days at 37°C in humidified 5%CO<sub>2</sub> as described in EXAMPLE 2. During incubation, 160  $\mu$ l of old medium in each well 50 was replaced with the same volume of the fresh medium containing test sample diluted with  $1 \times 10^{-8}$ M of activated vitamin D<sub>3</sub> and  $\alpha$ -MEM containing FBS on day 3 and day 5. On day 7, after washing the wells with phosphate buffered saline, cells were fixed with ethanol/acetone (1:1) for 1 min. and then osteoclast development was tested using acid phosphatase activity mesuring kit (Acid Phosphatase, Leucocyte, Catalog No. 387-A, Sigma Co.). The decrease of the number of TRAP positive cells was taken as an OCIF activity. As result, the conditioned medium showed the same OCIF activity as natural OCIF protein from IMR-90 conditioned medium (Table 4).

Table 4

OCIF activity of 293/EBNA conditioned medium.							
Cultured Cell	Dilution						
	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
OCIF expression vector transfected	++	++	++	++	++	+	-
vector transfected	-	-	-	-	-	-	-
untreated	-	-	-	-	-	-	-

[ ++ ; OCIF activity inhibiting osteoclast development more than 80%, + ; OCIF activity inhibiting osteoclast development between 30% and 80%, and - ; no OCIF activity. ]

## iii) Isolation of recombinant OCIF protein from 293/EBNA-conditioned medium

293/EBNA-conditioned medium (1.8 l) obtained by cultivating the cells described in example 13-ii) was supplemented with 0.1 % of CHAPS and filtrated with 0.22  $\mu$ m membrane filter (Steribecs GS, Millipore Co.). The conditioned medium was applied to 50 ml of a heparin Sepharose CL-6B column (2.6 x 10 cm, Pharmacia Co.) equilibrated with 10mM Tris-HCl, pH 7.5. After washing the column with 10mM Tris-HCl, pH 7.5, the adsorbed protein was eluted from the column with linear gradient from 0 to 2 M NaCl at a flow rate of 4 ml/min for 100 min. and fractions (8 ml) were collected. Using 150  $\mu$ l of each fraction, OCIF activity was assayed according to the method described in EXAMPLE 2. OCIF active fraction (112 ml) eluted with approximately 0.6 to 1.2 M NaCl was obtained.

One hundred twelve ml of the active fraction was diluted to 1000 ml with 10 mM Tris-HCl, 0.1% CHAPS, pH 7.5, and applied to a heparin affinity column (heparin-5PW, 0.8 x 7.5 cm, Tosoh Co.) equilibrated with 10mM Tris-HCl, 0.1% CHAPS, pH 7.5. After washing the column with 10mM Tris-HCl, 0.1% CHAPS, pH 7.5, the adsorbed protein was eluted from the column with linear gradient from 0 to 2 M NaCl at a flow rate of 0.5ml/min for 60 min., and fractions (0.5 ml) were collected. Four  $\mu$ l of each fraction was analyzed by SDS-polyacrylamide gel electrophoresis under reducing and non-reducing conditions as described in EXAMPLE 4. On SDS-PAGE under reducing conditions, a single band of rOCIF protein with an apparent 60 KD was detected in fractions from 30 to 32, under non-reducing conditions, bands of rOCIF protein with an apparent 60 KD and 120 KD were also detected in fractions from 30 to 32. The isolated rOCIF fraction from 30 to 32 was designated as recombinant OCIF derived from 293/EBNA (rOCIF(E)). 1.5 ml of the rOCIF(E) (535  $\mu$ g/ml) was obtained when determined by the method of Lowry using bovine serum albumin as a standard protein.

## EXAMPLE 14

## Production of recombinant OCIF using CHO cells

## i) Construction of the plasmid for expressing OCIF

pBKOCIF containing about 1.6 kb OCIF cDNA was prepared as described in EXAMPLE 11, and digested with restriction enzymes, Sall and EcoRV. About 1.4 kb OCIF cDNA insert was separated by an agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The expression vector, pcDL-SR  $\alpha$ 296 (Molecular and Cellular Biology, vol 8, p466, 1988) was digested with restriction enzymes, PstI and KpnI. About 3.4 kb of the expression vector fragment was cut out, separated by agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The ends of the purified OCIF cDNA insert and the expression vector fragment were blunted using DNA blunting kit (Takara Shuzo). The purified OCIF cDNA insert and the expression vector fragment were ligated using DNA ligation kit ver. 2 (Takara Shuzo). E.coli. DH5a  $\alpha$  (Gibco BRL) was transformed with the ligation mixture. The transformant containing the OCIF expression plasmid, pSR $\alpha$ OCIF was obtained.

## ii) Preparation of expression plasmid

The transformant containing the OCIF expression plasmid, pSR  $\alpha$ OCIF prepared in the example 13-i) and the transformant containing the mouse DHFR expression plasmid, pBAdDSV shown in WO92/01053 were grown according to the standard method. Both plasmids were purified by alkali treatment, polyethylene glycol precipitation, and cesium chrolide density gradient ultra centrifugation according to method of Maniatis et al. (Molecular cloning, 2nd edition).

## iii) Adaptation of CHOdhFr- cells to the protein free medium

CHOdhFr- cells (ATCC, CRL 9096) were cultured in IMDM containing 10 % fetal calf serum. The cells were adapted to EX-CELL 301 (JRH Bioscience) and then adapted to EX-CELL PF CHO (JRH Bioscience) according to the manufacturer's instructions.

## iv) Transfection of the OCIF expression plasmid, and the mouse DHFR expression plasmid, to CHOdhFr- cells.

CHOdhFr- cells prepared in EXAMPLE 14-iii) were transfected by electroporation with pSRαOCIF and pBAdDSV prepared in EXAMPLE 14-ii). 200 µg of pSRαOCIF and 20 µg of pBAdDSV were dissolved under sterile conditions in 0.8 ml of IMDM (Gibco BRL) containing 10 % fetal calf serum CG. 2x10<sup>7</sup> cells of CHOdhFr- were suspended in 0.8 ml of this medium. The cell suspension was transferred to a cuvette (Bio Rad) and the cells were transfected by electroporation using gene pulser (Bio Rad) under condition of 360 V and 960 µF. The suspension of electroporated cells was transferred to T-flasks (Sumitomo Bakelite) containing 10 ml of EX-CELL PF-CHO, and incubated in the CO<sub>2</sub> incubator for 2 days. Then the transfected cells were inoculated in each well of a 96 well plate (Sumitomo Bakelite) at a density of 5000 cells/well and cultured for about 2 weeks. The transformants expressing DHFR are selected since EX-CELL PF-CHO does not contain nucleotides and the parental cell line CHO dhFr- can not grow in this medium. Most of the transformants expressing DHFR express OCIF since the OCIF expression plasmid was used ten times as much as the mouse DHFR expression plasmid. The transformants whose conditioned medium had high OCIF activity were selected among the transformants expressing DHFR according to the method described in EXAMPLE 2. The transformants that express large amounts of OCIF were cloned by limiting dilution. The clones whose conditioned medium had high OCIF activity were selected as described above and the transformant expressing large amount of OCIF, 5561, was obtained.

## v) Production of recombinant OCIF

To produce recombinant OCIF (rOCIF), EX-CELL 301 medium (3 l) in a 3 l-spiner flask was inoculated with the clone (5561) at a cell-density of 1x10<sup>5</sup> cells/ml. The 5561 cells were cultured in a spinner flask at 37°C for 4 to 5 days. When the concentration of the 5561 cells reached to 1x10<sup>6</sup> cells/ml, about 2.7 l of the conditioned medium was harvested. Then about 2.7 l of EX-CELL 301 was added to the spinner flask and the 5561 cells were cultured repeatedly. About 20 l of the conditioned medium was harvested using the three spinner flasks.

## vi) Isolation of recombinant OCIF protein from CHO cells-conditioned medium

CHOcells-conditioned medium (1.0 l) described in EXAMPLE 14-v) was supplemented with 1.0 g of CHAPS and filtrated with 0.22 µm membrane filter (Steribucks GS, Milipore Co.). The conditioned medium was applied to a heparin Sepharose-FF column (2.6 x 10 cm, Pharmacia Co.) equilibrated with 10 mM Tris-HCl, pH 7.5. After washing the column with 10 mM Tris-HCl, 0.1 % CHAPS, pH 7.5, the adsorbed protein was eluted from the column with linear gradient from 0 to 2 M NaCl at a flow rate of 4 ml/min for 100 min. and fractions (8 ml) were collected. Using 150 µl of each fraction, OCIF activity was assayed according to the method described in EXAMPLE 2. Active fraction (112 ml) eluted with approximately 0.6 to 1.2 M NaCl was obtained.

The 112 ml of active fraction was diluted to 1200 ml with 10 mM Tris-HCl, 0.1% CHAPS, pH 7.5, and applied to a affinity column (blue-5PW, 0.5 x 5.0 cm, Tosoh Co.) equilibrated with 10 mM Tris-HCl, 0.1% CHAPS, pH 7.5. After washing the column with 10 mM Tris-HCl, 0.1% CHAPS, pH 7.5, the adsorbed protein was eluted from the column with linear gradient from 0 to 3 M NaCl at a flow rate of 0.5ml/min for 60 min., and fractions (0.5 ml) were collected. Four µl of each fraction was subjected to SDS-polyacrylamide gel electrophoresis under reducing and non-reducing conditions as described in EXAMPLE 4. On SDS-PAGE under reducing conditions, a single band of rOCIF protein with apparent 60 KD was detected in fractions 30 to 38, under non-reducing conditions, bands of rOCIF protein with apparent 60 KD and 120 KD were also detected in fractions 30 to 38. The isolated rOCIF fraction, 30 to 38, was designated as purified recombinant OCIF derived from CHO cells (rOCIF(C)). 4.5 ml of the rOCIF(C) (113 µg/ml) was obtained when determined by the method of Lowry using bovine serum albumin as a standard protein.

## EXAMPLE 15

## Determination of N-terminal amino acid sequence of rOCIFs

Each 3 µg of the isolated rOCIF(E) and rOCIF(C) was adsorbed to polyvinylidene difluoride (PVDF) membranes with Prospin (PERKIN ELMER Co.). The membranes were washed with 20 % ethanol and the N-terminal amino acid sequences of the adsorbed proteins were analyzed by protein sequencer (PROCISE 492, PERKIN ELMER Co.). The

determined N-terminal amino acid sequence is shown in sequence No. 7.

The N-terminal amino acid of rOCIF(E) and rOCIF(C) was the 22th amino acid of glutamine from Met as translation starting point, as shown in sequence number 5. The 21 amino acids from Met to Gln were identified as a signal peptide. The N-terminal amino acid sequence of OCIF isolated from IMR-90 conditioned medium was undetectable. Accordingly, the N-terminal glutamine of OCIF may be blocked by converting from glutamine to pyroglutamine within culturing or purifying.

#### EXAMPLE 16

##### 10 Biological activity of recombinant(r) OCIF and natural(n) OCIF

###### i) Inhibition of vitamin D<sub>3</sub> induced osteoclast formation from murine bone marrow cells

Each the rOCIF(E) and nOCIF sample was diluted with  $\alpha$ -MEM (GIBCO BRL Co.) containing 10% FBS and  $2 \times 10^{-8}$ M of activated vitamin D<sub>3</sub> (final concentration of 250 ng/ml). Each sample was serially diluted with the same medium, and 100  $\mu$ l of each diluted sample was added to each well in 96-well plates. Bone marrow cells obtained from mice, 17 days-old, were inoculated at a cell density of  $3 \times 10^5$  cells/100  $\mu$ l/ well to each well in 96-well plates and cultured for 7 days at 37°C in humidified 5%CO<sub>2</sub>. On day 7, the cells were fixed and stained with a acid phosphatase measuring kit (Acid Phosphatase, Leucocyte, No387-A, Sigma) according to the method described in EXAMPLE 2. The decrease of acid phosphatase activity (TRAP) was taken as OCIF activity. The decrease of acid phosphatase-positive cells was evaluated by solubilizing the pigment of dye and measuring absorbance. In detail, 100  $\mu$ l of a mixture of 0.1 N NaOH and dimethylsulfoxide (1:1) was added to each well and the well was vibrated to solubilize the dye. After solubilizing the dye completely, an absorbance of each well was measured at 590 nm subtracting the absorbance at 490 nm using microplate reader (Immunoreader NJ-2000, InterMed). The microplate reader was adjusted to 0 absorbance using a well with monolayered bone marrow cells which was cultured in the medium without activated vitamin D<sub>3</sub>. The decrease of TRAP activity was expressed as a percentage of the control absorbance value (=100%) of the solubilized dye from wells with bone marrow cells which were cultured in the absence of OCIF. The results are shown in Table 5.

30 Table 5

Inhibition of vitamin D <sub>3</sub> -induced osteoclast formation from murine bone marrow cells						
OCIF concentration(ng/ml)	250	125	63	31	16	0
rOCIF(E)	0	0	3	62	80	100
nOCIF	0	0	27	27	75	100 (%)

40 Both nOCIF and rOCIF(E) inhibited osteoclast formation in a dose dependent manner in the concentration of 16 ng/ml or higher

###### ii) Inhibition of vitamin D<sub>3</sub>-induced osteoclast formation in co-cultures of stromal cells and mouse spleen cells.

45 Effect of OCIF on osteoclast formation induced by Vitamin D<sub>3</sub> in co-cultures of stromal cells and mouse spleen cells was tested according to the method of N. Udagawa et al. (Endocrinology, vol. 125, p1805-1813, 1989). In detail, each of rOCIF(E), rOCIF(C), and nOCIF sample was serially diluted with  $\alpha$ -MEM (GIBCO BRL Co.) containing 10% FBS,  $2 \times 10^{-8}$ M of activated vitamin D<sub>3</sub>, and  $2 \times 10^{-7}$ M dexamethasone, and 100  $\mu$ l of each of the diluted samples was added to each well in 96 well-microwell plates. Murine bone marrow-derived stromal ST2 cells (RIKEN Cell Bank RCB0224) ; 50  $5 \times 10^3$  cells per 100  $\mu$ l of  $\alpha$ -MEM containing 10% FBS, and spleen cells from ddY mice, 8 weeks-old, ;  $1 \times 10^5$  cells per 100  $\mu$ l in the same medium, were inoculated to each well in 96-well plates and cultured for 5 days at 37°C in humidified 5%CO<sub>2</sub>. On day 5, the cells were fixed and stained with a kit for acid phosphatase (Acid Phosphatase, Leucocyte, No387-A, Sigma). The decrease of acid phosphatase-positive cells was taken as OCIF activity. The decrease of acid phosphatase-positive cells was evaluated according to the method described in EXAMPLE 16-i). The results are shown in Table 6 ; rOCIF(E) and rOCIF(C), and Table 7 ; rOCIF(E) and nOCIF.

Table 6

Inhibition of osteoclast formation in co-cultures of stromal cells and mouse spleen cells.					
OCIF concentration(ng/ml)	50	25	13	6	0
rOCIF(E)	3	22	83	80	100
rOCIF(C)	13	19	70	96	100 (%)

Table 7

Inhibition of osteoclast formation in co-cultures of stromal cells and mouse spleen cells.				
OCIF concentration(ng/ml)	250	63	16	0
rOCIF(E)	7	27	37	100
rOCIF(C)	13	23	40	100 (%)

nOCIF, rOCIF(E) and rOCIF(C) inhibited osteoclast formation in a dose dependent manner in the concentration of 6 - 16 ng/ml or higher

## iii) Inhibition of PTH-induced osteoclast formation from murine bone marrow cells.

Effect of OCIF on osteoclast formation induced by PTH was tested according to the method of N. Takahashi et al. (Endocrinology, vol. 122, p1373-1382, 1988). In detail, each the rOCIF(E) and nOCIF sample (125 ng/ml) was serially diluted with  $\alpha$ -MEM (manufactured by GIBCO BRL Co.) containing 10% FBS and  $2 \times 10^{-8}$ M PTH, and 100 $\mu$ l of each the diluted samples was added to 96 well-plates. Bone marrow cells from ddY mice, 17 days-old, at a cell density of  $3 \times 10^5$  cells per 100 $\mu$ l of  $\alpha$ -MEM containing 10% FBS were inoculated to each well in 96-wells plates and cultured for 5 days at 37°C in humidified 5%CO<sub>2</sub>. On day 5, the cells were fixed with ethanol/aceton (1:1) for 1 min. at room temperature and stained with a kit for acid phosphatase (Acid Phosphatase, Leucocyte, No387-A, Sigma) according to the method described in EXAMPLE 2. The decrease of acid phosphatase-positive cells was taken as OCIF activity. The decrease of acid phosphatase-positive cells was evaluated according to the method described in EXAMPLE 16-i). The results are shown in Table 8.

Table 8

Inhibition of PTH-induced osteoclast formation from murine bone marrow cells.						
OCIF concentration(ng/ml)	125	63	31	16	8	0
rOCIF(E)	6	58	58	53	88	100
nOCIF	18	47	53	56	91	100

nOCIF and rOCIF(E) inhibited osteoclast formation in a dose dependent manner in the concentration of 16 ng/ml or higher

## iv) Inhibition of IL-11-induced osteoclast formation

Effect of OCIF on osteoclast formation induced by IL-11 was tested according to the method of T. Tamura et al. (Proc. Natl. Acad. Sci. USA, vol. 90, p11924-11928, 1993). In detail, each rOCIF(E) and nOCIF sample was serially

diluted with  $\alpha$ -MEM (GIBCO BRL Co.) containing 10% FBS and 20 ng/ml IL-11 and 100 $\mu$ l of each the diluted sample was added to each well in 96-well plates. Newborn mouse calvaria-derived pre-adipocyte MC3T3-G2/PA6 cells (RIKEN Cell Bank RCB1127) ; 5x10<sup>3</sup> cells per 100 $\mu$ l of  $\alpha$ -MEM containing 10% FBS, and spleen cells from ddY mouse, 8 weeks-old, ; 1x10<sup>5</sup> cells per 100  $\mu$ l in the same medium, were inoculated to each well in 96-well plates and cultured for 5 days at 37 °C in humidified 5%CO<sub>2</sub>. On day 5, the cells were fixed and stained with a kit for acid phosphatase (Acid Phosphatase, Leucocyte, No387-A, Sigma). Acid phosphatase positive cells were counted under microscope and a decrease of the cell numbers was taken as OCIF activity. The results are shown in Table 9.

Table 9

OCIF concentration(ng/ml)	500	125	31	7.8	2.0	0.5	0
nOCIF	0	0	1	4	13	49	31
rOCIF(E)	0	0	1	3	10	37	31
Both nOCIF and rOCIF(E) inhibited osteoclast formation in a dose dependent manner in the concentration of 2 ng/ml or higher							

The results shown in Table 4-8 indicated that OCIF inhibits all the vitamin D<sub>3</sub>, PTH, and IL-11-induced osteoclast formations at almost the same doses. Accordingly, OCIF would be able to be used for treatment of the different types of bone disorders with decreased bone mass, which are caused by different substances which induce bone resorption.

## EXAMPLE 17

## Isolation of monomer-type OCIF and dimer-type OCIF

Each rOCIF(E) and rOCIF(C) sample containing 100  $\mu$ g of OCIF protein, was supplemented with 1/100 volume of 25 % trifluoro acetic acid and applied to a reverse phase column (PROTEIN-RP, 2.0x250 mm, YMC Co.) equilibrated with 30 % acetonitrile containing 0.1 % trifluoro acetic acid. OCIF protein was eluted from the column with linear gradient from 30 to 55 % acetonitrile at a flow rate of 0.2 ml/min for 50 min. and each OCIF peak was collected. Each the monomer-type OCIF peak fraction and dimer-type OCIF peak fraction was lyophilized, respectively.

## EXAMPLE 18

## Determination of molecular weight of recombinant OCIFs

Each 1  $\mu$ g of the isolated monomer-type and dimer-type nOCIF purified using reverse phase column according to EXAMPLE 3-iv) and each 1  $\mu$ g of monomer-type and dimer-type rOCIF described in EXAMPLE 17 was concentrated under vaccum, respectively. Each sample was incubated in the buffer for SDS-PAGE, subjected to SDS-polyacrylamide gel electrophoresis, and protein bands on the gel were stained with silver according to the method described in EXAMPLE 4. Results of electrophoresis under non-reducing conditions and reducing conditions are shown in Figure 6 and Figure 7.

A protein band with an apparent molecular weight of 60 KD was detected in each monomer-type OCIF sample, and a protein band with an apparent molecular weight of 120 KD was detected in each dimer-type OCIF sample in non-reducing conditions. A protein band with an apparent molecular weight of 60 KD was detected in each monomer-type OCIF sample under reducing conditions. Accordingly, molecular weights of monomer-type nOCIF from IMR-90 cells, rOCIF from 293/EBNA cells and rOCIF from CHO cells were almost the same. Molecular weights of dimer-type nOCIF from IMR-90 cells, rOCIF from 293/EBNA cells, and rOCIF from CHO cells were also the same.

## EXAMPLE 19

## Remove N-linked Oligosaccharide chain and Mesuring molecular weight of natural and recombinant OCIF

Each sample containing 5 $\mu$ g of the isolated monomer-type and dimer-type nOCIF purified using reverse phase column according to EXAMPLE 3-iv) and each sample containing 5  $\mu$ g of monomer-type and dimer-type rOCIF described in EXAMPLE 17 were concentrated under vaccum. Each sample was dissolved in 9.5  $\mu$ l of 50 mM sodium phosphate buffer, pH 8.6, containing 100 mM 2-mercaptoethanol, supplemented with 0.5  $\mu$ l of 250 U/ml N-glycanase (Seikagaku

kogyo Co.) and incubated for one day at 37 °C. Each sample was supplemented with 10 µl of 20 mM Tris-HCl, pH 8.0 containing 2 mM EDTA, 5 % SDS, and 0.02 % bromo-phenol blue and heated for 5 min at 100 °C. Each 1 µl of the samples was subjected to SDS-polyacrylamide gel electrophoresis, and protein bands on the gel were stained with silver as described in EXAMPLE 4. The patterns of electrophoresis are shown in Figure 8.

5 An apparent molecular weight of each the deglycosylated nOCIF from IMR-90 cells, rOCIF from CHO cells, and rOCIF from 293/EBNA cells was 40 KD under reducing conditions. An apparent molecular weight of each untreated nOCIF from IMR-90 cells, rOCIF from 293/EBNA cells, and rOCIF from CHO cells was 60 KD under reducing conditions. Accordingly, the results indicate that the OCIF proteins are glycoproteins with N-linked sugar chains.

10 EXAMPLE 20

Cloning of OCIF variant cDNAs and determination of their DNA sequences

The plasmid pBKOCIF, which is inserted OCIF cDNA to pBKCMV (Stratagene), was obtained from one of some purified positive phage as in example 10 and 11. And more, during the screening of the cDNA library with the 397 bp OCIF cDNA probe, the transformants containing plasmids whose insert sizes were different from that of pBKOCIF were obtained. These transformants containing the plasmids were grown and the plasmids were purified according to the standard method. The sequence of the insert DNA in each plasmid was determined using Taq Dye Deoxy Terminator Cycle Sequencing kit (Perkin Elmer). The used primers were T3, T7 primers (Stratagene) and synthetic primers prepared based on the nucleotide sequence of OCIF cDNA. There are four OCIF variants (OCIF2, 3, 4, and 5) in addition to OCIF. The nucleotide sequence of OCIF2 is shown in the sequence number 8 and the amino acid sequence of OCIF 2 predicted by the nucleotide sequence is shown in the sequence number 9. The nucleotide sequence of OCIF3 is shown in the sequence number 10 and the amino acid sequence of OCIF3 predicted by the nucleotide sequence is shown in the sequence number 11. The nucleotide sequence of OCIF4 is shown in the sequence number 12 and the amino acid sequence of OCIF4 predicted by the nucleotide sequence is shown in the sequence number 13. The nucleotide sequence of OCIF5 is shown in the sequence number 14 and the amino acid sequence of OCIF5 predicted by the nucleotide sequence is shown in the sequence number 15. The structures of OCIF variants are shown in Figures 9 to 12 and are described in brief below. OCIF2

OCIF2 cDNA has a deletion of 21 bp from guanine at nucleotide number 265 to guanine at nucleotide number 285 in OCIF cDNA (sequence number 6). Accordingly OCIF2 has a deletion of 7 amino acids from glutamic acid (Glu) at amino acid number 68 to glutamine (Gln) at amino acid number 74 in OCIF (sequence number 5).

OCIF3

35 OCIF3 cDNA has a point mutation at nucleotide number 9 in OCIF cDNA (sequence number 6) where cytidine is replaced with guanine.

Accordingly OCIF3 has a mutation and asparagine (Asn) at amino acid number -19 in OCIF (sequence number 5) is replaced with lysine (Lys). The mutation seems to be located in the signal sequence and have no essential effect on the secreted OCIF3. OCIF3 cDNA has a deletion of 117 bp from guanine at nucleotide number 872 to cytidine at nucleotide number 988 in OCIF cDNA (sequence number 6).

Accordingly OCIF3 has a deletion of 39 amino acids from threonine (Thr) at amino acid number 270 to leucine (Leu) at amino acid number 308 in OCIF (sequence number 5).

OCIF4

45 OCIF4 cDNA has two point mutations in OCIF cDNA (sequence number 6). Cytidine at nucleotide number 9 is replaced with guanine and guanine at nucleotide number 22 is replaced with thymidine in OCIF cDNA (sequence number 6).

Accordingly OCIF4 has two mutations. Asparagine (Asn) at amino acid number -19 in OCIF (sequence number 5) is replaced with lysine (Lys), and alanine (Ala) at amino acid number -14 is replaced with serine (Ser). These mutations seem to be located in the signal sequence and have no essential effect on the secreted OCIF4.

OCIF4 cDNA has about 4 kb DNA, which is the intron 2 of OCIF gene, inserted between nucleotide number 400 and nucleotide number 401 in OCIF cDNA (sequence number 6). The open reading frame stops in intron 2.

Accordingly OCIF4 has an additional novel amino acid sequence containing 21 amino acids after alanine (Ala) at amino acid number 112 in OCIF (sequence number 5).

## OCIF5

OCIF5 cDNA has a point mutation at nucleotide number 9 in OCIF cDNA (sequence number 6) where cytidine is replaced with guanine.

5 Accordingly OCIF5 has a mutation and asparagine (Asn) at amino acid number -19 in OCIF (sequence number 5) is replaced with lysine (Lys). The mutation seems to be located in the signal sequence and have no essential effect on the secreted OCIF5.

OCIF5 cDNA has the latter portion (about 1.8 kb) of intron 2 between nucleotide number 400 and nucleotide number 401 in OCIF cDNA (sequence number 6). The open reading frame stops in the latter portion of intron 2.

10 Accordingly OCIF5 has an additional novel amino acid sequence containing 12 amino acids after alanine (Ala) at amino acid number 112 in OCIF (sequence number 5).

## EXAMPLE 21

## 15 Production of OCIF variants

## i) Construction of the plasmid for expressing OCIF variants

20 The plasmid containing OCIF2 or OCIF3 cDNA was obtained as described in EXAMPLE 20 and called pBKOCIF2 and pBKOCIF3, respectively. pBKOCIF2 and pBKOCIF3 were digested with restriction enzymes, BamHI and Xhol. The OCIF2 and OCIF3 cDNA inserts were separated by agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The purified OCIF2 and OCIF3 cDNA inserts were individually ligated using DNA ligation kit ver. 2 (Takara Shuzo) to the expression vector pCEP4 (Invitrogen) that had been digested with restriction enzymes, BamHI and Xhol. E. coli. DH5 $\alpha$  (Gibco BRL) was transformed with the ligation mixture.

25 The plasmid containing OCIF4 cDNA was obtained as described in EXAMPLE 20 and called pBKOCIF4. pBKOCIF4 was digested with restriction enzymes, SpeI and Xhol (Takara Shuzo). The OCIF4 cDNA insert was separated by an agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The purified OCIF4 cDNA insert was ligated using DNA ligation kit ver. 2 (Takara Shuzo) to the expression vector pCEP4 (Invitrogen) that had been digested with restriction enzymes, NheI and Xhol (Takara Shuzo). E.coli. DH5  $\alpha$  (Gibco BRL) was transformed with the ligation mixture.

30 The plasmid containing OCIF5 cDNA was obtained as described in EXAMPLE 20 and was called pBKOCIF5. pBKOCIF5 was digested with restriction enzyme, HindIII (Takara Shuzo). The 5' portion of the coding region in the OCIF5 cDNA insert was separated by agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The OCIF expression plasmid, pCEPOCIF, obtained in EXAMPLE 13-i) was digested with restriction enzyme, HindIII (Takara Shuzo). The 5' portion of the coding region in the OCIF cDNA was removed. The rest of the plasmid that contains pCEP vector and the 3' portion of the coding region of OCIF cDNA was called pCEPOCIF-3'. pCEPOCIF-3' was separated by an agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The OCIF5 cDNA HindIII fragment and pCEPOCIF-3' were ligated using DNA ligation kit ver. 2 (Takara Shuzo). E.coli. DH5  $\alpha$  (Gibco BRL) was transformed with the ligation mixture.

40 The obtained transformants were grown at 37 °C overnight and the OCIF variants expression plasmids (pCEPOCIF2, pCEPOCIF3, pCEPOCIF4, and pCEPOCIF5) were purified using QIAGEN column (QIAGEN). These OCIF-variants-expression plasmids were precipitated with ethanol, dissolved in sterile distilled water, and used in the experiments described below.

## 45 ii) Transient expression of OCIF variant cDNAs and analysis of the biological activity of recombinant OCIF variants.

50 Recombinant OCIF variants were produced using the expression plasmid, pCEPOCIF2, pCEPOCIF3, pCEPOCIF4, and pCEPOCIF5 prepared as described in EXAMPLE 21-i) according to the method described in EXAMPLE 13-ii). The biological activities of recombinant OCIF variants were analyzed. The results were that these OCIF variants (OCIF2, OCIF3, OCIF4, and OCIF5) had a weak activity.

## EXAMPLE 22

## Preparation of OCIF mutants

55 55 i) Construction of a plasmid vector for subcloning cDNAs encoding OCIF mutants

The plasmid vector (5  $\mu$ g) described in EXAMPLE 11 was digested with restriction enzymes Bam HI and Xho I (

Takara Shuzo). The digested DNA was subjected to a preparative agarose gel electrophoresis. DNA fragment with an approximate size of 1.6 kilobase pairs (kb) that contained the entire coding sequence for OCIF was purified from the gel using QIAEX gel extraction kit (QIAGEN). The purified DNA was dissolved in 20  $\mu$ l of sterile distilled water. This solution was designated DNA solution 1. p Bluescript II SK + (3  $\mu$ g) (Stratagene) was digested with restriction enzymes Bam HI and Xho I (Takara Shuzo). The digested DNA was subjected to preparative agarose gel electrophoresis. DNA fragment with an approximate size of 3.0 kb was purified from the gel using QIAEX DNA extraction kit (QIAGEN). The purified DNA was dissolved in 20  $\mu$ l of sterile distilled water. The solution was designated DNA solution 2. One microliter of DNA solution 2, 4  $\mu$ l of DNA solution 1 and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 (Takara Shuzo) were mixed and incubated at 16 °C for 30 min. (The ligation mixture was used for the transformation of *E. coli* in a manner described below). Conditions for transformation of *E. coli* were as follows. One hundred microliters of competent *E. coli* DH5  $\alpha$  cells (GIBCO BRL) and 5  $\mu$ l of the ligation mixture was mixed in a sterile 15-ml tube (IWAKI glass). The tube was kept on ice for 30 min. After incubation for 45 sec at 42°C, to the cells was added 250  $\mu$ l of L broth (1% Tryptone, 0.5% yeast extract, 1% NaCl). The cell suspension was then incubated for 1 hr. at 37°C with shaking. Fifty microliters of the cell suspension was plated onto an L-agar plate containing 50  $\mu$ g/ml of ampicillin. The plate was incubated overnight at 37°C.

Six colonies which grew on the plate were individually incubated in 2 ml each of L-broth containing 50  $\mu$ g/ml of ampicillin overnight at 37°C with shaking. The structure of the plasmids in the colonies was analyzed. A plasmid in which the 1.6-kb DNA fragment containing the entire OCIF cDNA is inserted between the digestion sites of Bam HI and Xho I of pBluescript II SK + was obtained and designated as pSK + -OCIF.

ii) Preparation of mutants in which one of the Cys residues in OCIF is replaced with Ser residue

1) Introduction of mutations into OCIF cDNA

OCIF mutants were prepared in which one of the five Cys residues present in OCIF at positions 174, 181, 256, 298 and 379 (in SEQUENCE NO 4) was replaced with Ser residue and were designated OCIF-C19S(174Cys to Ser), OCIF-C20S (181Cys to Ser), OCIF-C21S (256Cys to Ser), OCIF-C22S (298Cys to Ser) and OCIF-C23S (379Cys to Ser), respectively.

To prepare the mutants, nucleotides encoding the corresponding Cys residues were replaced with those encoding Ser. Mutagenesis was carried out by a two-step polymerase chain reaction (PCR). The first step of the PCRs consisted of two reactions, PCR 1 and PCR 2.

35	PCR 1	10X Ex Taq Buffer (Takara Shuzo)	10 $\mu$ l
		2.5 mM solution of dNTPs	8 $\mu$ l
		the plasmid vector described in EXAMPLE 11 (8ng/ml)	2 $\mu$ l
		sterile distilled water	73.5 $\mu$ l
		20 $\mu$ M solution of primer 1	5 $\mu$ l
	PCR 2	100 $\mu$ M solution of primer 2 (for mutagenesis)	1 $\mu$ l
		Ex Taq (Takara Shuzo)	0.5 $\mu$ l
		10X Ex Taq Buffer (Takara Shuzo)	10 $\mu$ l
		2.5 mM solution of dNTPs	8 $\mu$ l
		the plasmid vector described in EXAMPLE 11 (8ng/ml)	2 $\mu$ l
40	PCR 2	sterile distilled water	73.5 $\mu$ l
		20 $\mu$ M solution of primer 3	5 $\mu$ l
		100 $\mu$ M solution of primer 4 (for mutagenesis)	1 $\mu$ l
		Ex Taq (Takara Shuzo)	0.5 $\mu$ l
45			
50			

55 Specific sets of primers were used for each mutation and other components were unchanged. Primers used for the reactions are shown in Table 10. The nucleotide sequences of the primers are shown in SEQUENCE NO: 20,23,27 and 30-40. The PCRs were performed under the following conditions as follows. An initial denaturation step at 97°C for 3 min was followed by 25 cycles of denaturation at 95°C for 1 min annealing at 55°C for 1 min and extension at 72°C for

3 min. After these amplification cycles, final extension was performed at 70°C for 5 min. The size of the PCR products was confirmed by agarose gel electrophoresis using reaction solution. After the first PCR, excess primers were removed using Amicon microcon (Amicon). The final volume of the solutions that contained the PCR products were made to 50µl with sterile distilled water. These purified PCR products were used for the second PCR (PCR 3).

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PCR 3	10X Ex Taq Buffer (Takara Shuzo)	10 µl
	2.5 mM solution of dNTPs	8 µl
	solution containing DNA fragment obtained from PCR 1	5 µl
	solution containing DNA fragment obtained from PCR 2	5 µl
	sterile distilled water	61.5 µl
	20 µM solution of primer 1	5 µl
	20 µM solution of primer 3	5 µl
	Ex Taq (Takara Shuzo)	0.5 µl

25

30

Table 10

mutants	primer-1	primer-2	primer-3	primer-4
OCIF-C19S	IF 10	C19SR	IF 3	C19SF
OCIF-C20S	IF 10	C20SR	IF 3	C20SF
OCIF-C21S	IF 10	C21SR	IF 3	C21SF
OCIF-C22S	IF 10	C22SR	IF 14	C22SF
OCIF-C23S	IF 6	C23SR	IF 14	C23SF

The reaction conditions were exactly the same as those for PCR 1 or PCR 2. The size of the PCR products was confirmed by 1.0 % or 1.5 % agarose gel electrophoresis. The DNA fragments were precipitated with ethanol, dried under vacuum and dissolved in 40 µl of sterile distilled water. The solutions containing DNA fragments with mutation C19S, C20S, C21S, C22S and C23S were designated as DNA solution A, DNA solution B, DNA solution C, DNA solution D and DNA solution E, respectively.

The DNA fragment which is contained in solution A (20µl) was digested with restriction enzymes Nde I and Sph I (Takara Shuzo). A DNA fragment with an approximate size of 400 base pairs (bp) was extracted from a preparative agarose gel and dissolved in 20 µl of sterile distilled water. This DNA solution was designated DNA solution 3. Two micrograms of pSK + -OCIF was digested with restriction enzymes Nde I and Sph I. A DNA fragment with an approximate size of 4.2 kb was purified from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 µl of sterile distilled water. This DNA solution was designated as DNA solution 4. Two microliters of DNA solution 3, 3 µl of DNA solution 4 and 5 µl of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent *E. coli* DH5  $\alpha$  cells were transformed with 5 µl of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-C19S.

The DNA fragment which is contained in solution B (20 µl) was digested with restriction enzymes Nde I and Sph I. A DNA fragment with an approximate size of 400 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20µl of sterile distilled water. This DNA solution was designated DNA solution 5. Two microliters of DNA solution 5, 3 µl of DNA solution 4 and 5 µl of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent *E. coli* DH5  $\alpha$  cells were transformed with 5 µl of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-C20S.

The DNA fragment which is contained in solution C (20 µl) was digested with restriction enzymes Nde I and Sph I. A DNA fragment with an approximate size of 400 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20µl of sterile distilled water. This DNA solution was designated as DNA solution 6. Two micro-

liters of DNA solution 6, 3  $\mu$ l of DNA solution 4 and 5 $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent *E. coli* DH5  $\alpha$  cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-C21S.

5 The DNA fragment which is contained in solution D (20  $\mu$ l) was digested with restriction enzymes Nde I and Bst PI. A DNA fragment with an approximate size of 600 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20  $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 7. Two micrograms of pSK + -OCIF was digested with restriction enzymes Nde I and Bst PI. A DNA fragment with an approximate size of 4.0 kb was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 8. Two microliters of DNA solution 7, 3  $\mu$ l of DNA  
10 solution 8 and 5 $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent *E. coli* DH5  $\alpha$  cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA in which the 600-bp Nde I-BstPI fragment with the mutation (the C22S mutation) is substituted for the 600-bp Nde I-Bst PI fragment of pSK+ -OCIF by analyzing the DNA structure. DNA structure  
15 was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-C22S.

The DNA fragment which is contained in solution E (20  $\mu$ l) was digested with restriction enzymes Bst PI and Eco RV. A DNA fragment with an approximate size of 120 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 9. Two  
20 micrograms of pSK + -OCIF was digested with restriction enzymes Bst EII and Eco RV. A DNA fragment with an approximate size of 4.5 kb was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 10. Two microliters of DNA solution 9, 3  $\mu$ l of DNA solution 10 and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation was carried out. Competent  
25 *E. coli* DH5  $\alpha$  cells were transformed with 5 $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-C23S.

## 2) Construction of vectors for expressing the OCIF mutants

30 pSK-OCIF-C19S, pSK-OCIF-C20S, pSK-OCIF-C21S, pSK-OCIF-C22S and pSK-OCIF-C23S were digested with restriction enzymes Bam HI and Xho I. The 1.6 kb Bam HI-Xho I DNA fragment encoding each OCIF mutant was isolated and dissolved in 20 $\mu$ l of sterile distilled water. The DNA solutions that contain 1.6 kb cDNA fragments derived from pSK-OCIF-C19S, pSK-OCIF-C20S, pSK-OCIF-C21S, pSK-OCIF-C22S and pSK-OCIF-C23S were designated C19S DNA solution, C20S DNA solution, C21S DNA solution, C22S DNA solution and C23S DNA solution, respectively. Five  
35 micrograms of a expression vector pCEP 4 (Invitrogen) was digested with restriction enzymes Bam HI and Xho I. A DNA fragment with an approximate size of 10 kb was purified and dissolved in 40 $\mu$ l of sterile distilled water. This DNA solution was designated as pCEP 4 DNA solution. One microliter of pCEP 4 DNA solution and 6  $\mu$ l of either C19SDNA solution, C20S DNA solution, C21S DNA solution, C22S DNA solution or C23S DNA solution were independently mixed with 7  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 and ligation reactions were carried out. Competent *E. coli*  
40 DH5 $\alpha$  cells (100 $\mu$ l) were transformed with 7  $\mu$ l of each ligation mixture. Ampicillin-resistant transformants were screened for clones containing plasmid in which a 1.6-kb cDNA fragment is inserted between the recognition sites of Bam HI and Xho I of pCEP 4 by analyzing the DNA structure. The plasmide which were obtained containing the cDNA encoding OCIF-C19S, OCIF-C20S, OCIF-C21S, OCIF-C22S and OCIF-C23S were designated pCEP4-OCIF-C19S, pCEP4-OCIF-C20S, pCEP4-OCIF-C21S, pCEP4-OCIF-C22S and pCEP4-OCIF-C23S, respectively.

45 ii) Preparation of domain-deletion mutants of OCIF

### (1) deletion mutagenesis of OCIF cDNA

50 A series of OCIF mutants with deletions of from Thr 2 to Ala 42, from Pro 43 to Cys 84, from Glu 85 to Lys 122, from Arg 123 to Cys 164, from Asp 177 to Gln 251 and from Ile 252 to His 326 were prepared (positions of the amino acid residues are shown in SEQUENCE NO: 4). These mutants were designated as OCIF-DCR1, OCIF-DCR2, OCIF-DCR3, OCIF-DCR4, OCIF-DDD1 and OCIF-DDD2, respectively.

Mutagenesis was performed by two-step PCR as described in EXAMPLE 22-(ii). The primer sets for the reactions are  
55 shown in Table 11 and the nucleotide sequences of the primers are shown in SEQUENCE NO: 19, 25, 40-53, and 54.

Table 11

mutants	primer-1	primer-2	primer-3	primer-4
OCIF-DCR1	Xhol F	DCR1R	IF 2	DCR1F
OCIF-DCR2	Xhol F	DCR2R	IF 2	DCR2F
OCIF-DCR3	Xhol F	DCR3R	IF 2	DCR3F
OCIF-DCR4	Xhol F	DCR4R	IF 16	DCR4F
OCIF-DDD1	IF 8	DDD1R	IF 14	DDD1F
OCIF-DDD2	IF 8	DDD2R	IF 14	DDD2F

5 The final PCR products were precipitated with ethanol, dried under vacuum and dissolved in 40 $\mu$ l of sterile distilled water. Solutions of DNA fragment coding for portions of OCIF-DCR1, OCIF-DCR2, OCIF-DCR3, OCIF-DCR4, OCIF-DDD1 and OCIF-DDD2 were designated as DNA solutions F, G, H, I, J and K, respectively.

10 The DNA fragment which is contained in solution F (20  $\mu$ l) was digested with restriction enzymes Nde I and Xho I. A

20 DNA fragment with an approximate size of 500 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 $\mu$ l of sterile distilled water. This DNA solution was designated DNA solution 11. Two micrograms of pSK+ -OCIF was digested with restriction enzymes Nde I and Xho I. A DNA fragment with an approximate size of 4.0 kb was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 $\mu$ l of sterile distilled water. This DNA solution was designated DNA solution 12. Two microliters of DNA solution 11, 3  $\mu$ l of DNA solution 12 and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation was carried out. Competent E. coli

25 DH5  $\alpha$  cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DCR1.

The DNA fragment which is contained in solution G (20  $\mu$ l) was digested with restriction enzymes Nde I and Xho I.

30 A DNA fragment with an approximate size of 500 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 13. Two microliters of DNA solution 13, 3  $\mu$ l of DNA solution 12 and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation was carried out. Competent E. coli DH5a cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DCR2.

The DNA fragment which is contained in solution H (20  $\mu$ l) was digested with restriction enzymes Nde I and Xho I. A DNA fragment with an approximate size of 500 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 14. Two microliters of DNA solution 14, 3  $\mu$ l of DNA solution 12 and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and

40 ligation reaction was carried out. Competent E. coli DH5  $\alpha$  cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DCR3.

The DNA fragment which is contained in solution I (20  $\mu$ l) was digested with restriction enzymes Xho I and Sph I. A DNA fragment with an approximate size of 900 bp was extracted from a preparative agarose gel with QIAEX gel extraction

45 kit and dissolved in 20 $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 15. Two micrograms of pSK+ -OCIF was digested with restriction enzymes Xho I and Sph I. A DNA fragment with an approximate size of 3.6 kb was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 16. Two microliters of DNA solution 15, 3  $\mu$ l of DNA solution 16 and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent E. coli DH5  $\alpha$  cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DCR4.

The DNA fragment which is contained in solution J (20  $\mu$ l) was digested with restriction enzymes BstP I and Nde I. A DNA fragment with an approximate size of 400 bp was extracted from a preparative agarose gel with QIAEX gel extraction

55 kit and dissolved in 20  $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 17. Two microliters of DNA solution 17, 3  $\mu$ l of DNA solution 8 and 5 $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent E. coli DH5  $\alpha$  cells were transformed with 5 $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by

restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DDD1. The DNA fragment which is contained in solution K (20  $\mu$ l) was digested with restriction enzymes Nde I and BstP I. A DNA fragment with an approximate size of 400 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20  $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 18. Two microliters of DNA solution 18, 3  $\mu$ l of DNA solution 8 and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent *E. coli* DH5  $\alpha$  cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DDD2.

10 2) Construction of vectors for expressing the OCIF mutants

pSK-OCIF-DCR1, pSK-OCIF-DCR2, pSK-OCIF-DCR3, pSK-OCIF-DCR4, pSK-OCIF-DDD1 and pSK-OCIF-DDD2 were digested with restriction enzymes Bam HI and Xho I. The Bam HI-Xho I DNA fragment containing entire coding sequence for each OCIF mutant was isolated and dissolved in 20  $\mu$ l of sterile distilled water. These DNA solutions that 15 contain the Bam HI-Xho I fragment derived from pSK-OCIF-DCR1, pSK-OCIF-DCR2, pSK-OCIF-DCR3, pSK-OCIF-DCR4, pSK-OCIF-DDD1 and pSK-OCIF-DDD2 were designated DCR1 DNA solution, DCR2 DNA solution, DCR3 DNA solution, DCR4 DNA solution, DDD1 DNA solution and DDD2 DNA solution, respectively. One microliter of pCEP 4 DNA solution and 6  $\mu$ l of either DCR1 DNA solution, DCR2 DNA solution, DCR3 DNA solution, DCR4 DNA solution, DDD1 DNA solution or DDD2 DNA solution were independently mixed with 7  $\mu$ l of ligation buffer I of DNA ligation kit ver. 20 2 and ligation reactions were carried out. Competent *E. coli* DH5 $\alpha$  cells (100  $\mu$ l) were transformed with 7  $\mu$ l of each ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA in which the DNA 25 fragment with deletions is inserted between the recognition sites of Bam HI and Xho I of pCEP 4 by analyzing the DNA structure. The plasmids containing the cDNA encoding OCIF-DCR1, OCIF-DCR2, OCIF-DCR3, OCIF-DCR4, OCIF-DDD1 and OCIF-DDD2 were designated as pCEP4-OCIF-DCR1, pCEP4-OCIF-DCR2, pCEP4-OCIF-DCR3, pCEP4-OCIF-DCR4, pCEP4-OCIF-DDD1 and pCEP4-OCIF-DDD2, respectively.

iii) Preparation of OCIF with C-terminal domain truncation

(1) mutagenesis of OCIF cDNA

30 A series of OCIF mutants with deletions of from Cys at amino acid residue 379 to Leu 380, from Ser 331 to Leu 380, from Asp 252 to Leu 380, from Asp 177 to Leu 380, from Arg 123 to Leu 380 and from Cys 86 to Leu 380 was prepared. Positions of the amino acid residues are shown in SEQUENCE NO: 4. These mutants were designated as OCIF-CL, OCIF-CC, OCIF-CDD2, OCIF-CDD1, OCIF-CCR4 and OCIF-CCR3, respectively.

35 Mutagenesis for OCIF-CL was performed by the two-step PCR as described in EXAMPLE 22-(ii). The primer set for the reaction is shown in Table 12. The nucleotide sequences of the primers are shown in SEQUENCE NO:23, 40, 55, and 56. The final PCR products were precipitated with ethanol, dried under vacuum and dissolved in 40  $\mu$ l of sterile distilled water. This DNA solution was designated as solution L.

The DNA fragment which is contained in solution L (20  $\mu$ l) was digested with restriction enzymes BstP I and EcoR V. A DNA fragment with an approximate size of 100 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20  $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 19. Two microliters of DNA solution 19, 3  $\mu$ l of DNA solution 10 (described in EXAMPLE 22-(ii)) and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent *E. coli* DH5  $\alpha$  cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA 45 structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-CL. Mutagenesis of OCIF cDNA to prepare OCIF-CC, OCIF-CDD2, OCIF-CDD1, OCIF-CCR4 and OCIF-CCR3 was performed by a one-step PCR.

PCR reactions for mutagenesis to prepare OCIF-CC, OCIF-CDD2, OCIF-CDD1, OCIF-CCR4 and OCIF-CCR3

50

55

5	10X Ex Taq Buffer (Takara Shuzo)	10 $\mu$ l
	2.5 mM solution of dNTPs	8 $\mu$ l
	the plasmid vector containing the entire OCIF cDNA described in EXAMPLE 11 (8ng/ml)	2 $\mu$ l
	sterile distilled water	73.5 $\mu$ l
10	20 $\mu$ M solution of primer OCIF Xho F	5 $\mu$ l
	100 $\mu$ M solution of primer (for mutagenesis)	1 $\mu$ l
	Ex Taq (Takara Shuzo)	0.5 $\mu$ l

15

Table 12

mutants	primer-1	primer-2	primer-3	primer-4
OCIF-CL	IF 6	CL R	IF 14	CL F

Specific primers were used for each mutagenesis and other components were unchanged.

Primers used for the mutagenesis are shown in Table 13. Their nucleotide sequences are shown in SEQUENCE NO:57-61. The components of each PCR were mixed in a microcentrifuge tube and PCR was performed as follows. The microcentrifuge tubes were treated for 3 minutes at 97 °C and then incubated sequentially, for 30 seconds at 95 °C, 30 seconds at 50 °C and 3 minutes at 70 °C. This three-step incubation procedure was repeated 25 times, and after that, the tubes were incubated for 5 minutes at 70 °C. An aliquot of the reaction mixture was removed from each tube and analyzed by an agarose gel electrophoresis to confirm the size of each product.

The size of the PCR products was confirmed on an agarose gel. Excess primers in the PCRs were removed using Amicon microcon (Amicon) after completion of the reaction. The DNA fragments were precipitated with ethanol, dried under vacuum and dissolved in 40  $\mu$ l of sterile distilled water. The DNA fragment in each DNA solution was digested with restriction enzymes Xho I and Bam HI. After the reactions, DNA was precipitated with ethanol, dried under vacuum and dissolved in 20  $\mu$ l of sterile distilled water.

The solutions containing DNA fragment with the CC deletion, the CDD2 deletion, the CDD1 deletion, the CCR4 deletion and the CCR3 deletion were designated as CC DNA solution, CDD2 DNA solution, CDD1 DNA solution, CCR4 DNA solution and CC R3 DNA solution, respectively.

Table 13

mutants	primers for the mutagenesis
OCIF-CC	CC R
OCIF-CDD2	CDD2 R
OCIF-CDD1	CDD1 R
OCIF-CCR4	CCR4 R
OCIF-CCR3	CCR3 R

## (2) Construction of vectors for expressing the OCIF mutants

pSK-OCIF-CL was digested with restriction enzymes Bam HI and Xho I. The Bam HI-Xho I DNA fragment containing the entire coding sequence for OCIF-CL was isolated and dissolved in 20  $\mu$ l of sterile distilled water. This DNA solution was designated as CL DNA solution. One microliter of pCEP 4 DNA solution and 6  $\mu$ l of either of CL DNA solution, CC DNA solution, CDD2 DNA solution, CDD1 DNA solution, CCR4 DNA solution or CCR3 DNA solution were independently mixed with 7  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 and ligation reactions were carried out. Competent

E. coli DH5 $\alpha$  cells (100  $\mu$ l) were transformed with 7  $\mu$ l of each ligation mixture. Ampicillin-resistant transformants were screened for clones containing plasmids which have the desirable mutations in OCIF cDNA by analyzing the DNA structure. In each plasmid, OCIF cDNA fragment having a deletion were inserted between the recognition sites of Xho I and Bam HI of pCEP 4. The plasmids containing the cDNA encoding OCIF-CL, OCIF-CC, OCIF-CDD1, OCIF-CDD2, OCIF-  
5 CCR4 and OCIF-CCR3 were designated pCEP4-OCIF-CL, pCEP4-OCIF-CC, pCEP4-OCIF-CDD2, pCEP4-OCIF-CDD1, pCEP4-OCIF-CCR4 and pCEP4-OCIF-CCR3, respectively.

iv) Preparation of OCIF mutants with C-terminal truncation

10 (1) Introduction of C-terminal truncation to OCIF

A series of OCIF mutants with C-terminal truncation was prepared. OCIF mutant in which 10 residues of from Gln at 371 to Leu at 380 are replaced with 2 residues of Leu-Val was designated OCIF-CBst. OCIF mutant in which 83 residues of from Cys 298 to Leu 380 are replaced with 3 residues of Ser-Leu-Asp was designated OCIF-CSph. OCIF mutant in which 214 residues of from Asn 167 to Leu 380 are removed was designated OCIF-CBsp. OCIF mutant in which 319 residues of from Asp 62 to Leu 380 are replaced with 2 residues of Leu-Val was designated OCIF-CPst. Positions of the amino acid residues are shown in SEQUENCE NO: 4.

20 Two micrograms each of pSK + -OCIF was digested with one of the restriction enzymes, Bst PI, Sph I, PstI (Takara Shuzo), and Bsp EI (New England Biolabs), and followed by phenol extraction and ethanol precipitation. The precipitated DNA was dissolved in 10  $\mu$ l of sterile distilled water. Ends of the DNAs in 2  $\mu$ l of each solution were blunted using a DNA blunting kit in final volumes of 5  $\mu$ l. To the reaction mixtures, 1  $\mu$ g (1  $\mu$ l) of an Amber codon-containing Xba I linker (5'-CTAGTCTAGACTAG-3') and 6  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were added.

25 After the ligation reactions, 6  $\mu$ l each of the reaction mixtures was used to transform E. coli DH5 $\alpha$ . Ampicillin-resistant transformants were screened for clones containing plasmids. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmids thus obtained were named pSK-OCIF-CBst, pSK-OCIF-CSph, pSK-OCIF-CBsp and pSK-OCIF-CPst, respectively.

(2) Construction of vectors for expressing the OCIF mutants

30 pSK-OCIF-CBst, pSK-OCIF-CSph, pSK-OCIF-CBsp and pSK-OCIF-CPst were digested with restriction enzymes Bam HI and Xho I. The 1.5 kb of DNA fragment containing entire coding sequence for each OCIF mutant was isolated and dissolved in 20  $\mu$ l of sterile distilled water. These DNA solutions that contain the Bam HI-Xhol fragment derived from pSK-OCIF-CBst, pSK-OCIF-CSph, pSK-OCIF-CBsp and pSK-OCIF-CPst were designated as CBst DNA solution, CSph DNA solution, CBsp DNA solution and CPst DNA solution, respectively. One microliter of pCEP 4 DNA solution (described in EXAMPLE 22-ii)) and 6  $\mu$ l of either CBst DNA solution, CSph DNA solution, CBsp DNA solution or CPst DNA solution were independently mixed with 7  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 and ligation reactions were carried out. Competent E. coli DH5 $\alpha$  cells (100  $\mu$ l) were transformed with 7  $\mu$ l of each ligation mixture. Ampicillin-resistant transformants were screened for clones containing plasmids in which cDNA fragment is inserted between the recognition sites of Bam HI and Xho I of pCEP 4 by analyzing the DNA structure. The plasmids containing the cDNA 35 encoding OCIF-CBst, OCIF-CSph, OCIF-CBsp and OCIF-CPst were designated as pCEP4-OCIF-CBst, pCEP4-OCIF-CSph, pCEP4-OCIF-CBsp and pCEP4-OCIF-CPst, respectively.

v) Preparation of vectors for expressing the OCIF mutants

40 45 E. coli clones harboring the expression vectors for OCIF mutants (total of 21 clones) were grown and the vectors were purified by QIAGEN column (QIAGEN). All the expression vectors were precipitated with ethanol and dissolved in appropriate volumes of sterile distilled water and used for further manipulations shown below.

50 vi) Transient expression of the cDNAs for OCIF mutants and biological activities of the mutants

55 OCIF mutants were produced using the expression vectors prepared in EXAMPLE 22-v). The method was essentially the same as described in EXAMPLE 13. Only the modified points are described below. A 24-well plate was used for the DNA transfection. 2X10<sup>5</sup> cells of 293/EBNA suspended in IMDM containing 10% fetal bovine serum were seeded into each well of the plate. One microgram of purified vector DNA and 4  $\mu$ l of lipofectamine were used for each transfection. Mixture of an expression vector and lipofectamine in OPTI-MEM (GIBCO BRL) in a final volume of 0.5 ml was added to the cells in a well. After the cells were incubated at 37°C for 24 hr in a CO<sub>2</sub> incubator, the medium was replaced with 0.5 ml of Ex-cell 301 medium (JSR). The cells were incubated at 37 °C for 48 more hours in the CO<sub>2</sub> incubator. The conditioned medium was collected and used for assay for in vitro biological activity. The nucleotide

sequences of cDNAs for the OCIF mutants are shown in SEQUENCE NO:83-103. The deduced amino acid sequences for the OCIF mutants are shown in SEQUENCE NO: 62-82. The assay for in vitro biological activity was performed as described in EXAMPLE 13. Antigen concentration of each conditioned medium was determined by ELISA as described in EXAMPLE 24. Table 14 shows specific activity of the mutants relative to that of the unaltered OCIF.

5

Table 14

	mutants	activity
10	the unaltered OIF	++
15	OCIF-C19S	+
20	OCIF-C20S	±
25	OCIF-C21S	±
30	OCIF-C22S	+
35	OCIF-C23S	++
40	OCIF-DCR1	±
	OCIF-DCR2	±
	OCIF-DCR3	±
	OCIF-DCR4	±
	OCIF-DDD1	+
	OCIF-DDD2	±
	OCIF-CL	++
	OCIF-CC	++
	OCIF-CDD2	++
	OCIF-CDD1	+
	OCIF-CCR4	±
	OCIF-CCR3	±
	OCIF-CBst	++
	OCIF-CSph	++
	OCIF-CBsp	±
	OCIF-CPst	±
++ indicates relative activity more than 50% of that of the unaltered OCIF + indicates relative activity between 10% and 50% ± indicates relative activity less than 10%, or production level too low to determine the accurate biological activity		

45

#### vii) western blot analysis

Ten microliters of the final conditioned medium was used for western blot analysis. Ten microliters of the sample were mixed with 10  $\mu$ l of SDS-PAGE sample buffer (0.5 M Tris-HCl, 20% glycerol, 4% SDS, 20 $\mu$ g/ml bromo phenol blue, pH 6.8) boiled for 3 min. and subjected to a 10 % SDS polyacryl amide gel electrophoresis under non-reducing conditions. After the electrophoresis, the separated proteins were blotted to PVDF membrane (ProBlott<sup>R</sup>, Perkin Elmer) using a semi-dry electroblotter (BIO-RAD). The membrane was incubated at 37°C with horseradish peroxidase labeled anti-OCIF antibodies for 2 hr. After the membrane was washed, protein bands which react with the labeled antibodies were detected using ECL system (Amersham). Two protein bands with approximate molecular masses of 60kD and 120kD were detected for the unaltered OCIF. On the other hand, almost exclusively 60kD protein band was detected for OCIF-C23S, OCIF-CL and OCIF CC. Protein bands with an approximate masses of 40kD-50kD and 30kD-40kD were the major ones for OCIF-CDD2 and OCIF-CDD1, respectively. These results indicate that Cys at 379 is responsible for the dimer formation, both the monomers and the dimers maintain the biological activity and a deletion of residues from Asp

at 177 to Leu at 380 does not abolish the biological activity of OCIF (positions of the amino acid resare shown in SEQUENCE NO: 4).

## EXAMPLE 23

5

Isolation of human genomic OCIF gene

i) Screening of a human genomic library

10 An amplified human placenta genomic library in Lambda FIX II vector purchased from STRATAGENE was screened for the gene encoding human OCIF using the human OCIF cDNA as a probe. Essentially, screening was done according to the instruction manual supplied with the genomic library. The basic protocols described in Molecular Cloning: A Laboratory Manual also were employed to manipulate phage, E. coli, and DNA.

15 The library was titered, and  $1 \times 10^6$  pfu of phage was mixed with XL1-Blue MRA host E. coli cells and plated on 20 plates (9 cm x 13 cm) with 9 ml per plate of top agarose. The plates were incubated overnight at 37°C. Filter plaque lifts were prepared using Hybond-N nylon membranes (Amersham). The membranes were processed by denaturation in a solution containing 1.5 M NaCl and 0.5 M NaOH for 1 minute at room temperature. The membranes were then neutralized by placing successively for one minute each in 1 M Tris-HCl (pH7.5) and a solution containing 1.5 M NaCl and 0.5 M Tris-HCl (pH 7.5). The membranes were then transferred onto a filter paper wet with 2xSSC. Phage DNA was fixed 20 on the membranes with 1200  $\mu$ Joules of UV energy in STRATALINKER UV crosslinker 2400 (STRATAGENE) and the membranes were air dried. The membranes were immersed in Rapid Hybridization buffer (Amersham) and incubated for one hour at 65 °C before hybridization with  $^{32}$ P-labeled cDNA probe in the same buffer overnight at 65°C. Screening probe was prepared by labeling the OCIF cDNA with  $^{32}$ P using the Megaprime DNA labeling system (Amersham). Approximately,  $5 \times 10^5$  cpm probe was used for each ml of hybridization buffer. After the hybridization, the membranes 25 were rinsed in 2xSSC for five minutes at room temperature. The membranes were then washed four times, 20 minutes each time, in 0.5xSSC containing 0.1 % SDS at 65 °C. After the final wash, the membranes were dried and subjected to autoradiography at -80 °C with SUPER HR-H X-ray film (FUJI PFOTO FILM Co., Ltd.) and an intensifying screen. Upon examination of the autoradiograms, six positive signals were detected. Agar plugs were picked from the regions 30 corresponded to these signals for phage purification. Each agar plug was soaked overnight in 0.5 ml of SM buffer containing 1% chloroform to extract phage. Each extract containing phage was diluted 1000 fold with SM buffer and an aliquot of 1 ml or 20 ml was mixed with host E. coli described above. The mixture was plated on agar plates with top agarose as described above. The plates were incubated overnight at 37 °C, and filter lifts were prepared, prehybridized, hybridized, washed and autoradiographed as described above. This process of phage purification was applied to all six 35 positive signals initially detected on the autoradiograms and was repeated until all phage plaques on agar plates hybridize with the cDNA probe. After purification, agar plugs of each phage isolate were soaked in SM buffer containing 1% chloroform and stored at 4 °C. Six individual phage isolates were designated  $\lambda$ OIF3,  $\lambda$ OIF8,  $\lambda$ OIF9,  $\lambda$ OIF11,  $\lambda$ OIF12 and  $\lambda$ OIF17, respectively.

ii) Analysis of the genomic clones by restriction enzyme digestion and Southern blot hybridization

40

DNA was prepared from each phage isolate by the plate lysate method as described in Molecular Cloning: A Laboratory Manual. DNA prepared from each phage was digested with restriction enzymes and the fragments derived from the digestion were separated on agarose gels. The fragments were then transferred to nylon membranes and subjected to Southern blot hybridization using OCIF cDNA as a probe. The results of the analysis revealed that the six phage isolates 45 are individual clones. Among these fragments derived from the restriction enzyme digestion, those fragments which hybridized with the OCIF cDNA probe were subcloned into plasmid vectors and subjected to the nucleotide sequence analysis as described below.

iii) Subcloning restriction fragments derived from genomic clones into plasmid vectors and determination of the nucleotide sequence.

$\lambda$ OIF8 DNA was digested with restriction enzymes EcoRI and NotI, and the DNA fragments derived these from were separated on a 0.7% agarose gel. The 5.8 kilobase pairs (kb) EcoRI/NotI fragment was extracted from the gel using QIAEX II Gel Extraction Kit (QIAGEN) according to the procedure recommended by the manufacturer. The 5.8 kb 55 EcoRI/NotI fragment was ligated with pBluescript II SK+ vector (STRATAGENE) which had been linearized with restriction enzymes EcoRI and NotI, using Ready-To-Go T4 DNA Ligase (Pharmacia) according to the procedure recommended by the manufacturer. Competent DH5  $\alpha$  E. coli cells (Amersham) were transformed with the recombinant plasmid and transformants were selected on L-plates containing 50  $\mu$ g/ml of ampicillin. A clone harboring the recom-

5 binant plasmid containing the 5.8 kb EcoRI/NotI fragment was isolated and this plasmid was termed pBSG8-5.8. pBSG8-5.8 was digested with HindIII and 0.9 kb of DNA fragment derived from this digestion was isolated in the same manner as described above. This 0.9 kb fragment was then cloned in pBluescript II SK- at the HindIII site as described above. This recombinant plasmid containing 0.9 kb HindIII fragment was denoted pBS8H0.9.

10 5  $\lambda$ OIF11 DNA was digested with EcoRI and 6 kb, 3.6 kb, 2.6 kb EcoRI fragments were isolated in the same manner as described above and cloned in pBluescript II SK+ vector at the EcoRI site as described above. These recombinant plasmids were termed pBSG11-6, pBSG11-3.6, and pBSG11-2.6, respectively. pBSG11-6 was digested with HindIII and the digest was applied on a 0.7 % agarose gel. Three fragments, 2.2 kb, 1.1 kb, and 1.05 kb in length, were extracted from the gel and cloned independently in pBluescript II SK- vector at the HindIII site in the same manner as described above. These recombinant plasmids were termed pBS6H2.2, pBS6 H1.1 and pBS6H1.05, respectively.

15 10 The nucleotide sequence of the cloned genomic DNA was determined using ABI Dyedeoxy Terminator Cycle Sequencing Ready Reaction Kit (PERKIN ELMER) and 373A DNA Sequencing system (Applied Biosystems). Plasmids pBSG8-5.8, pBS8H0.9, pBSG11-6, pBSG11-3.6, pBSG11-2.6, pBS6H2.2, pBS6H1.1 and pBS6H1.05 were prepared according to the alkaline-SDS procedure as described in Molecular Cloning: A Laboratory Manual and used as 15 templates for the DNA sequence analysis. Nucleotide sequence of the human OCIF gene was presented in Sequence No 104 and Sequence No 105. The nucleotide sequence of the DNA, between exon 1 and exon 2 was not entirely determined. There is a stretch of approximately 17 kb of nucleotides between the sequences given in sequence No. 104 and sequence No. 105.

20 EXAMPLE 24

Quantitation of OCIF by EIA

i) Preparation of anti-OCIF antibody

25 25 Male JW rabbits (Kitayama LABES Co. ,LTD) weighing 2.5-3.0 kg were used for immunization for preparing antisera. Three male JW rabbits (Kitayama LABES Co., LTD) weighing 2.5-3.0 kg were used for immunization. For immunization, emulsion was prepared by mixing an equal volume of rOCIF (200  $\mu$ g/ml) and complete Freund's adjuvant (Difco, Cat. 0638-60-7). The rabbits were immunized subcutaneously six times at the interval of one week with 1 ml of emulsion 30 per injection. The rabbits were injected six times at the interval of seven days subcutaneously. Whole blood was obtained ten days after the final immunization and serum was separated. Antibody was purified from serum as follows. Antiserum was diluted two-fold with PBS. After adding ammonium sulfate at a final concentration of 40 w/v %, antiserum was allowed to stand at 4 °C for 1 hr.. Precipitate obtained by centrifugation at 8000 x g for 20 min. was dissolved in a small volume of PBS and was dialyzed against PBS. The resulting solution was loaded onto a Protein G-Sepharose column (Pharmacia). After washing with PBS, absorbed immunoglobulin G was eluted with 0.1 M glycine-HCL buffer (pH 3.0). Elutes were neutralized with 1.5 M Tris-HCL buffer (pH 8.7) immediately and were dialyzed against PBS. Protein concentration was determined by absorbance at 280nm (E<sup>1%</sup> 13.5).

35 40 Horseradish peroxidase labeled antibody was prepared using ImmunoPure Maleimide Activated Horseradish Peroxidase Kit (Pierce, Cat. 31494). Briefly, one mg of IgG was incubated with 80 ug of N-succinimidyl-S-acetylthioacetate for 30 min. After deacetylation with 5 mg of hydroxylamine HCl, modified IgG was separated by polyacrylamide desalting column. Protein pool mixed with one mg of maleimide activated horseradish peroxidase was incubated at room temperature for 1 hr.

ii) Quantitation of OCIF by sandwich EIA

45 50 Microtiter plates (Nunc MaxiSorp Immunoplate) were coated with rabbit anti-OCIF IgG by incubating 0.2 ug in 100 ul of 50 mM sodium bicarbonate buffer pH 9.6 at 4C overnight. After blocking the plates by incubating for 1 hour at 37°C with 300 ul of 25% BlockAce/PBS (Snow Brand Milk Products ), 100ul of samples were incubated for 2 hours at room temperature. After washing the plates three times with PBST (PBS containing 0.05% Tween20), 100 ul of 1:10000 diluted horseradish peroxidase labeled anti-OCIF IgG was added and incubated for 2 hours at room temperture. The amount of OCIF was determined by incubation with 100 ul of a substrate solution (TMB, ScyTek Lab., Cat. TM4999) and measurement of the absorbance at 450 nm using an ImmunoReader (Nunc NJ2000). Purified recombinant OCIF was used as a standard protein and a typical standard curve was shown in Fig. 13.

## EXAMPLE 25

## Anti-OCIF monoclonal antibody

## 5 i) Preparation of hybridoma producing anti-OCIF monoclonal antibody.

OCIF was purified to homogeneity from culture medium of human fibroblasts, IMR-90 by the purification method described in Example 11. Purified OCIF was dissolved in PBS at a concentration of 10 µg/100 µl. BALB/c mice were immunized by administrating this solution intraperitoneally three times every two weeks. In the first and the second 10 immunizations, the emulsion composed of an equal volume of OCIF and Freund's complete adjuvant was administered. Three days after the final administration, the spleen was taken out, lymphocytes were isolated and fused with mouse myeloma p3x63-Ag8.653 cells according to the conventional method using polyethyleneglycol. Then the fused cells were cultured in HAT medium to select hybridoma. Subsequently, to check whether the selected hybridomas produce anti-OCIF antibody, anti-OCIF antibody in each culture medium of hybridomas was determined by solid phase ELISA which 15 was prepared by coating each well in 96-well immunoplates (Nunc) with 100µl of purified OCIF (10µg/ml in 0.1 M NaHCO<sub>3</sub>) and by blocking each well with 50% BlockAce (Snow Brand Milk Products Co. Ltd.). The hybridoma clones secreting anti-OCIF antibody were established by cloning 3 - 5 times by limit dilution and by screening using the above solid phase ELISA. Among thus obtained hybridoma clones, several hybridoma clones with high production of anti-OCIF antibody were selected.

## 20 ii) Production of anti-OCIF monoclonal antibodies.

Each hybridoma clone secreting anti-OCIF antibody, which was obtained in EXAMPLE 25-i), was transplanted intraperitoneally to mice given Pristane (Aldrich) at a cell density of 1 x 10<sup>6</sup> cells/mouse. The accumulated ascites was collected 25 10 - 14 days after the transplantation and the ascites containing anti-OCIF specific monoclonal antibody of the present invention was obtained. Purified antibodies were obtained by Affigel protein A Sepharose chromatography (BioRad) according to the manufacturer's manual. That is, the ascites was diluted with equal volume of a binding buffer (BioRad) and applied to protein A column. The column was washed with a sufficient volume of the binding buffer and eluted with an elution buffer (BioRad). After neutralizing, the obtained eluate was dialyzed in water and subsequently lyophilized. 30 The purity of the obtained antibody was analyzed by SDS/PAGE and a homogenous band with a molecular weight of about 150,000 was detected.

## iii) Selection of monoclonal antibody having high affinity to OCIF

35 Each antibody obtained in EXAMPLE 25-ii) was dissolved in PBS and the concentration of protein in the solution was determined by the method of Lowry. Each antibody solution with the same concentration was prepared and then serially diluted with PBS. Monoclonal antibodies, which can recognize OCIF even at highly diluted solution, were selected by solid phase ELISA described in EXAMPLE 25-ii). Thus three monoclonal antibodies A1G5, E3H8 and D2F4 can be selected.

## 40 iv) Determination of class and subclass of antibodies

The class and subclass of the antibodies of the present invention obtained in EXAMPLE 25-iii) were analyzed using an immunoglobulin class and subclass analysis kit (Amersham). The procedure was carried out according to the protocol disclosed in the directions. The results were shown in Table 15. The antibodies of the present invention, E3H8, 45 A1G5 and D2F4 belong to IgG<sub>1</sub>, IgG<sub>2a</sub> and IgG<sub>2b</sub>, respectively.

Table 15

Analysis of class and subclass of the antibodies in the present invention.							
Antibody	IgG <sub>1</sub>	IgG <sub>2a</sub>	IgG <sub>2b</sub>	IgG <sub>3</sub>	IgA	IgM	κ
A1G5	-	+	-	-	-	-	+
E3H8	+	-	-	-	-	-	+
D2F4	-	-	+	-	-	-	+

## v) Determination of OCIF by ELISA

Three kinds of monoclonal antibodies, A1G5, E3H8 and D2F4, which were obtained in EXAMPLE 25-iv), were used as solid phase antibodies and enzyme-labeled antibodies, respectively. Sandwich ELISA was constructed by each combination of solid phase antibody and labeled antibody. The labeled antibody was prepared using Immuno Pure Maleimide Activated Horseradish Peroxidase Kit (Pierce, Cat. No. 31494). Each monoclonal antibody was dissolved in 0.1 M NaHCO<sub>3</sub> at a concentration of 10 µg/ml, and 100 µl of the solution was added to each well in 96-well immunoplates (Nunc, MaxiSorp Cat. No. 442404) followed by allowing to stand at room temperature overnight. Subsequently, each well in the plates was blocked with 50% Blockace (Snow Brand Milk Products, Co. , Ltd. ) at room temperature for 50 minutes, and then was washed three times with PBS containing 0.1% Tween 20 (washing buffer).

10 A series of concentrations of OCIF was prepared by diluting OCIF with 1st reaction buffer (0.2 M Tris-HCl bufer, pH 7.4, containing 40% Blockace and 0.1% Tween 20). Each well in 96-well immunoplates was filled with 100µl of the prepared OCIF solution with each concentration, allowed to stand at 37 °C for 3 hours, and subsequently washed three times with the washing buffer. For dilution of POD-labeled antibody, 2nd reaction buffer (0.1 M Tris-HCl buffer, pH 7.4, containing 25% Blockace and 0.1% Tween 20) was used. POD-labeled antibody was diluted 400-fold with 2nd reaction buffer, and 100 µl of the diluted solution was added to each well in the immunoplates. Each imunoplate was allowed to stand at 37 °C for 2 hours, and subsequently washed three times with the washing buffer. After washing, 100 µl of a substrate solution (0.1 M citrate-phosphate buffer, pH 4. 5, containing 0.4 mg/ml of o-phenylenediamine HCl and 0.006% H<sub>2</sub>O<sub>2</sub>) was added to each well in the immunoplates and the immunoplates were incubated at 37°C for 15 min.

15 20 The enzyme reaction was terminated by adding 50 µl of 6 N H<sub>2</sub>SO<sub>4</sub> to each well. The optical density of each well was determined at 492 nm using an immunoreader (ImmunoReader NJ 2000, Nunc).

25 Using three kinds of monoclonal antibody in the present invention, each combination of solid phase and POD-labeled antibodies leads to a accurate determination of OCIF. Each monoclonal antibody in the present invention was confirmed to recognize a different epitope of OCIF. A typical standard curve of OCIF using a combination of solid phase antibody, A1G5 and POD-labeled antibody, E3H8 was shown in Fig. 14.

## vi) Determination of OCIF in human serum

30 Concentration of OCIF in five samples of normal human serum was determined using an EIA system described in EXAMPLE 25-v). The immunoplates were coated with A1G5 as described in EXAMPLE 25-v), and 50 µl of 1st. reaction buffer was added to each well in the immunoplates. Subsequently, 50µl of each human serum was added to each well in the immunoplates. The immnuoplates were incubated at 37°C for 3 hours and then washed three times with the washing buffer. After washing, each well in the immunoplates was filled with 100µl of POD-E3H8 antibody diluted 400-fold with 2nd. reaction buffer and incubated at 37°C for 2 hours. After washing the immunoplates three times with the washing buffer, 100 µl of the substrate solution described in EXAMPLE 25-v) was added to each well and incubated at 37°C for 15 min. The enzyme reaction was terminated by adding 50 µl of 6 N H<sub>2</sub>SO<sub>4</sub> to each well in the immunoplates. The optical density of each well was determined at 492 nm using an immunoreader (ImmunoReader NJ 2000, Nunc). 1st. reaction buffer containing the known amount of OCIF was treated in the same way and a standard curve of OCIF as shown in fig. 2 was obtained. Using the standard curve of OCIF, the amount of OCIF in human serum sample was 35 40 determined. The results were shown in Table 14.

Table 14

The amount of OCIF in normal human serum	
Serum Sample	OCIF Concentration (ng/ml)
1	5.0
2	2.0
3	1.0
4	3.0
5	1.5

EXAMPLE 26

Therapeutic effect on osteoporosis

5 (1) Method

Male Fischer rats, 6 weeks-old, were subjected to denervation of left forelimb. These rats were assigned to four groups(10 rats/group) and treated as follows ; group A, sham operated rats without administration ; group B, denervated rats with intravenous administration of vehicle ; group C, denervated rats administered OCIF intravenously at a dose of 10 5 µg/kg twice a day ; group D, denervated rats administered OCIF intravenously at a dose of 50 µg/kg twice a day. After denervation, OCIF was administered daily for 14 days. After 2 weeks treatment, the animals were sacrificed and their forelimbs were dissected. Thereafter bones were tested for mechanical strength.

(2) Results

15 Decrease of bone strength was observed in the animals of control groups as compared to those animals of the normal groups while bone strength was increase in the groups of animal received 50 mg of OCIF per kg body weight.

Industrial availability

20 The present invention provides both a novel protein which inhibits formation of osteoclasts and a efficient procedure to produce the protein. The protein of the present invention has an activity to inhibit formation of osteoclasts. The protein will be useful for the treatment of many diseases accompanying bone loss, such as osteoporosis, and as an antigen to be used for the immunological diagnosis of such diseases.

25 Referring to the deposited the microorganism

Name and Address of the Depositary Authority

30 Name: National Institute of Bioscience and Human-Technology Agency of Industrial Science and Technology Ministry of International Trade and Industry  
Address: 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken 305, JAPAN  
Deposited date: June 21, 1995  
(It was transferred from Bikkoken No. P-14998, which was deposited on June 21, 1995.

35 Transferred date: October 25, 1995)

Acession Number: FERM BP-5267

40

45

50

55

SEQUENCE LISTING

5 (1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: SNOW BRANDS MILK PRODUCTS CO., LTD.

(B) STREET:

10 (C) CITY:

(D) STATE:

(E) COUNTRY:

15 (F) POSTAL CODE (ZIP):

(G) TELEPHONE:

(H) TELEFAX:

20 (I) TELEX:

(ii) TITLE OF INVENTION: Novel proteins and methods for producing the  
proteins

(iii) NUMBER OF SEQUENCES: 105

25 (iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER:

30 (C) OPERATING SYSTEM:

(D) SOFTWARE: Wordperfect windows

(v) CURRENT APPLICATION DATA:

35 (A) APPLICATION NUMBER: JP

(B) FILE REFERENCE:

(C) FILING DATE:

40

45

50

55

(2) INFORMATION FOR SEQUENCE ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 6

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide (an internal amino acid sequence of the protein)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 1:

Xaa Tyr His Phe Pro Lys

1

5

(2) INFORMATION FOR SEQUENCE ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 14

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide (an internal amino acid sequence of the protein)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO:2:

Xaa Gln His Ser Xaa Gln Glu Gln Thr Phe Gln Leu Xaa Lys

1

5

10

(2) INFORMATION FOR SEQUENCE ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 12

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide (an internal amino acid sequence of the protein)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 3:

Xaa Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys

1

5

10

(2) INFORMATION FOR SEQUENCE ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 380



5	Lys Asp Gln Asp Ile Val Lys Lys Ile Gln Asp Ile Asp Leu		
	245	250	255
	Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr		
	260	265	270
10	Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys		
	275	280	285
	Val Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro		
	290	295	300
15	Ser Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn		
	305	310	315
	Gly Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His		
	320	325	330
20	Ser Lys Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys		
	335	340	345
	Lys Thr Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr		
	350	355	360
25	Gln Lys Leu Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val		
	365	370	375
30	Lys Ile Ser Cys Leu		
	380		

(2) INFORMATION FOR SEQUENCE ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 401

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF protein with signal peptide)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 5:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser

-20 -15 -10

Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys

-5 -1 1 5

Asp Glu Glu Thr Ser His Gln Leu Leu Cy

15 20

Gly Thr Tyr Leu Lys Glu His Cys Thr Ala Lys Trp Lys Thr

30 35

S - Al - R - Cys - Pro - Asp - His - Tyr - Tyr - Thr - Asp - Ser - Trp - His

Cys  $\alpha$  Ile Cys Ile Asp His Tyr Tyr Ile

	40	45	50
5	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
	55	60	65
	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
	70	75	80
10	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
	85	90	95
	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
	100	105	110
15	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
	115	120	125
	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
20	130	135	140
	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
	145	150	155
	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
25	160	165	170
	Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
	175	180	185
30	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
	190	195	200
	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
	205	210	215
35	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
	220	225	230
	Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
40	235	240	245
	Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
	250	255	260
	Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
45	265	270	275
	Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
	280	285	290
50	Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
	295	300	305
	Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		

310	315	320	
5	Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr		
325	330	335	
	Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe		
10	340	345	350
	Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly		
15	355	360	365
	Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu		
	370	375	380

## (2) INFORMATION FOR SEQUENCE ID NO: 6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1206
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : cDNA (OCIF)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 6:

30	ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60
	CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120
	TGTGACAAAT GTCCCTCCTGG TACCTACCTA AAACAAACACT GTACAGCAAA GTGGAAGACC 180
35	GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240
	CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300
	CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360
40	CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGA GCGAAATACA 420
	GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCCTGT 480
	AGAAAACACA CAAATTGCAAG TGTCTTTGGT CTCTGCTAA CTCAGAAAGG AAATGCAACA 540
	CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600
45	CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCCTACAA AGTTTACGCC TAACTGGCTT 660
	AGTGTCTTGG TAGACAATT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720
	AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780
	AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840
50	GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900
	AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960
	CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020

5 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080  
 GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCCCTCACA GCTTCACAAT GTACAAATTG 1140  
 TATCAGAAGT TATTTTTAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTGC 1200  
 TTATAA 1206

10 (2) INFORMATION FOR SEQUENCE ID NO: 7:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 15
- (B) TYPE : amino acid
- (D) TOPOLOGY : linear

20 (ii) MOLECULE TYPE : peptide (a N-terminal amino acid sequence of the protein)

25 (xi) SEQUENCE DESCRIPTION :SEQ ID NO:7:

Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser

1 5 10 15

25 (2) INFORMATION FOR SEQUENCE NO ID NO: 8:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1185
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

35 (ii) MOLECULE TYPE : cDNA (OCIF2)

35 (xi) SEQUENCE DESCRIPTION :SEQ ID NO:8

40 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240  
 CTATACTGCA GCCCCGTGTG CAAGGAGTGC AATCGCACCC ACAACCGCGT GTGCGAATGC 300  
 AAGGAAGGGC GCTACCTTGA GATAGAGTTC TGCTTGAAAC ATAGGAGCTG CCCTCCTGGA 360  
 TTTGGAGTGG TGCAAGCTGG AACCCCAGAG CGAAATACAG TTTGCAAAG ATGTCCAGAT 420  
 GGGTTCTTCT CAAATGAGAC GTCATCTAAA GCACCCCTGTA GAAAACACAC AAATTGCAGT 480  
 GTCTTTGGTC TCCTGCTAAC TCAGAAAGGA AATGCAACAC ACGACAACAT ATGTTCCGGA 540  
 AACAGTGAAT CAACTCAAAA ATGTGGAATA GATGTTACCC TGTGTGAGGA GGCATTCTTC 600  
 AGGTTTGCTG TTCCCTACAAA GTTACGCCT AACTGGCTTA GTGTCTTGGT AGACAATTG 660  
 CCTGGCACCA AAGTAAACGC AGAGAGTGTGAGAGGATAA AACGGCAACA CAGCTCACAA 720

5 GAACAGACTT TCCAGCTGCT GAAGTTATGG AAACATCAAA ACAAAGACCA AGATATAGTC 780  
 AAGAAGATCA TCCAAGATAT TGACCTCTGT GAAAACAGCG TGCAGCGGCA CATTGGACAT 840  
 GCTAACCTCA CCTTCGAGCA GCTTCGTAGC TTGATGGAAA GCTTACCGGG AAAGAAAGTG 900  
 GGAGCAGAAG ACATTGAAAA ACAATAAAG GCATGCAAAC CCAGTGACCA GATCCTGAAG 960  
 CTGCTCAGTT TGTGGCGAAT AAAAAATGGC GACCAAGACA CCTTGAAGGG CCTAATGCAC 1020  
 10 GCACTAAAGC ACTCAAAGAC GTACCACHTT CCCAAAATG TCACTCAGAG TCTAAAGAAG 1080  
 ACCATCAGGT TCCTTCACAG CTTCAAAATG TACAAATTGT ATCAGAAGTT ATTTTAGAA 1140  
 ATGATAGGTA ACCAGGTCCA ATCAGTAAAA ATAAGCTGCT TATAA 1185

## 15 (2) INFORMATION FOR SEQUENCE ID NO: 9:

## (i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH : 394  
 (B) TYPE : amino acid  
 (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : protein (OCIF2)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

25 Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser  
 -20 -15 -10  
 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
 -5 -1 1 5  
 30 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro  
 10 15 20  
 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr  
 25 30 35  
 35 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His  
 40 45 50  
 40 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Cys  
 45 55 60 65  
 Asn Arg Thr His Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr  
 70 75 80  
 45 Leu Glu Ile Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly  
 85 90 95  
 Phe Gly Val Val Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys  
 100 105 110  
 50 Lys Arg Cys Pro Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys  
 115 120 125

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Ala Pro Cys Arg Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu  
130 135 140  
5 Leu Thr Gln Lys Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly  
145 150 155  
Asn Ser Glu Ser Thr Gln Lys Cys Gly Ile Asp Val Thr Leu Cys  
160 165 170  
10 Glu Glu Ala Phe Phe Arg Phe Ala Val Pro Thr Lys Phe Thr Pro  
175 180 185  
Asn Trp Leu Ser Val Leu Val Asn Leu Pro Gly Thr Lys Val  
190 195 200  
15 Asn Ala Glu Ser Val Glu Arg Ile Lys Arg Gln His Ser Ser Gln  
205 210 215  
20 Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn Lys  
220 225 230  
Asp Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys  
235 240 245  
25 Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr Phe  
250 255 260  
Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val  
265 270 275  
30 Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser  
280 285 290  
Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly  
295 300 305  
35 Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser  
310 315 320  
Lys Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys  
325 330 335  
40 Thr Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln  
340 345 350  
45 Lys Leu Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys  
355 360 365  
Ile Ser Cys Leu  
50 370 373

(2) INFORMATION FOR SEQUENCE ID NO: 10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1089
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : cDNA (OCIF3)

## (xi) SEQUENCE DESCRIPTION ID NO: 10:

ATGAACAAGT	TGCTGTGCTG	CGCGCTCGTG	TTTCTGGACA	TCTCCATTAA	GTGGACCACC	60
CAGGAAACGT	TTCCCTCCAAA	GTACCTTCAT	TATGACGAAG	AAACCTCTCA	TCAGCTGTTG	120
TGTGACAAAT	GTCCTCCTGG	TACCTACCTA	AAACAACACT	GTACAGCAAA	GTGGAAGACC	180
GTGTGCGCCC	CTTGCCCTGA	CCACTACTAC	ACAGACAGCT	GGCACACCAG	TGACGAGTGT	240
CTATACTGCA	GCCCCGTGTG	CAAGGAGCTG	CAGTACGTCA	AGCAGGAGTG	CAATCCGACC	300
CACAACCGCG	TGTGCGAATG	CAAGGAAGGG	CGCTACCTTG	AGATAGAGTT	CTGCTTGAAA	360
CATAGGAGCT	GCCCTCCTGG	ATTTGGAGTG	GTGCAAGCTG	GAACCCCAGA	GCGAAATACA	420
GTGGCAAAA	GATGTCCAGA	TGGGTTCTTC	TCAAATGAGA	CGTCATCTAA	AGCACCCCTGT	480
AGAAAACACA	CAAATTGCAG	TGTCTTGTT	CTCCTGCTAA	CTCAGAAAGG	AAATGCAACA	540
CACGACAACA	TATGTTCCGG	AAACAGTGAA	TCAACTCAA	AATGTGGAAT	AGATGTTACC	600
CTGTGTGAGG	AGGCATTCTT	CAGGTTTGCT	GTTCTACAA	AGTTTACGCC	TAACGGCTT	660
AGTGTCTTGG	TAGACAATT	GCCTGGCACC	AAAGTAAACG	CAGAGAGTGT	AGAGAGGATA	720
AAACGGCAAC	ACAGCTCACA	AGAACAGACT	TTCCAGCTGC	TGAAGTTATG	GAAACATCAA	780
AACAAAGACC	AAGATATAGT	CAAGAAGATC	ATCCAAGATA	TTGACCTCTG	TGAAAACAGC	840
GTGCAGCGGC	ACATTGGACA	TGCTAACCTC	AGTTGTGGC	GAATAAAAAA	TGGCGACCAA	900
GACACCTTGA	AGGGCCTAAT	GCACGCACTA	AAGCACTCAA	AGACGTACCA	CTTTCCCAA	960
ACTGTCACTC	AGAGTCTAAA	GAAGACCATC	AGGTTCTTC	ACAGCTTCAC	AATGTACAAA	1020
TTGTATCAGA	AGTTATTTT	AGAAATGATA	GGTAACCAGG	TCCAATCAGT	AAAAATAAGC	1080
TGCTTATAA						1089

## (2) INFORMATION FOR SEQUENCE ID NO: 11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 362
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : protein (OCIF3)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Asn Lys Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser

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	-20	-15	-10
5	Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
	-5	-1 1	5
10	Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
	15	15	20
15	Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
	25	30	35
20	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Asp Ser Trp His		
	40	45	50
25	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
	55	60	65
30	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
	70	75	80
35	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
	85	90	95
40	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
	100	105	110
45	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
	115	120	125
50	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
	130	135	140
55	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
	145	150	155
60	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
	160	165	170
65	Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
	175	180	185
70	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
	190	195	200
75	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
	205	210	215
80	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
	220	225	230
85	Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
	235	240	245
90	Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		

250	255	260
5	Gly His Ala Asn Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln	
265	270	275
	Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr	
10	280	285
	285	290
Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile		
15	295	300
	300	305
Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu		
310	315	320
Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser		
325	330	335
Cys Leu		
20	340 341	

## (2) INFORMATION FOR SEQUENCE ID NO: 12:

## (i) SEQUENCE CHARACTERISTICS:

25	(A) LENGTH : 465	
	(B) TYPE : nucleic acid	
30	(C) STRANDEDNESS : single	
	(D) TOPOLOGY : linear	
35	(ii) MOLECULE TYPE : cDNA (OCIF4)	
	(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 12:	
40	ATGAACAAAGT TGCTGTGCTG CTCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60	
	CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120	
	TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180	
	GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGAGTGT 240	
45	CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300	
	CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360	
	CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GTACGTGTCA ATGTGCAGCA 420	
	AAATTAATTA GGATCATGCA AAGTCAGATA GTTGTGACAG TTTAG 465	

## (2) INFORMATION FOR SEQUENCE ID NO: 13:

## (i) SEQUENCE CHARACTERISTICS:

50	(A) LENGTH :154	
	(B) TYPE : amino acid	

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

5 (ii) MOLECULE TYPE : protein (OCIF4)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Met Asn Lys Leu Leu Cys Cys Ser Leu Val Phe Leu Asp Ile Ser  
-20 -15 -0  
10 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
-5 -1 1 5  
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro  
15 10 15 20  
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr  
25 25 30 35  
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His  
20 40 45 50  
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu  
55 55 60 65  
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys  
25 70 75 80  
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys  
30 85 90 95  
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr  
100 100 105 110  
Cys Gln Cys Ala Ala Lys Leu Ile Arg Ile Met Gln Ser Gln Ile  
35 115 120 125  
Val Val Thr Val  
130 133

40 (2) INFORMATION FOR SEQUENCE ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 438

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

45 (ii) MOLECULE TYPE : cDNA (OCIF5)

(xi) SEQUENCE DESCRIPTION ID NO: 14:

50 ATGAAACAAGT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60

5 CAGGAAACGT TTCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCACCTGTTG 120  
 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240  
 CTATACTGCA GCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 10 CACAACCGCG TGTGCAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GATGCAGGAG AAGACCCAAG 420  
 CCACAGATAT GTATCTGA 438

## (2) INFORMATION FOR SEQUENCE ID NO: 15:

## 15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 140
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : protein (OCIF5)

## (xi) SEQUENCE DESCRIPTION: ID NO: 15:

25 Met Asn Lys Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser  
 -20 -15 -10  
 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
 -5 -1 1 5  
 30 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro  
 10 15 20  
 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr  
 25 30 35  
 35 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His  
 40 45 50  
 40 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu  
 55 60 65  
 45 Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys  
 70 75 80  
 50 Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys  
 85 90 95  
 55 His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Cys  
 100 105 110  
 60 Arg Arg Arg Pro Lys Pro Gln Ile Cys Ile  
 115 120 124

(2) INFORMATION FOR SEQUENCE ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

10 (ii) MOLECULE TYPE : synthetic DNA (primer T3)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

AATTAACCCT CACTAAAGGG

20

15

(2) INFORMATION FOR SEQUENCE ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH : 22
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

25 (ii) MOLECULE TYPE : synthetic DNA (primer T7)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GTAATACGAC TCACTATAGG GC

22

30

(2) INFORMATION FOR SEQUENCE ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

40 (ii) MOLECULE TYPE : synthetic DNA (primer IF1)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 18:

ACATCAAAAC AAAGACCAAG

20

45

(2) INFORMATION FOR SEQUENCE ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- 50 (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

55

5 (ii) MOLECULE TYPE : synthetic DNA (primer IF2)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 19:

TCTTGGTCTT TGTTTGATG

20

10 (2) INFORMATION FOR SEQUENCE ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

15 (ii) MOLECULE TYPE : synthetic DNA (primer IF3)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 20:

20 TTATTCGCCA CAAACTGAGC

20

(2) INFORMATION FOR SEQUENCE ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

25 (ii) MOLECULE TYPE : synthetic DNA (primer IF4)

30 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 21:

35 TTGTGAAGCT GTGAAGGAAC

20

(2) INFORMATION FOR SEQUENCE ID NO: 22:

40 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

45 (ii) MOLECULE TYPE : synthetic DNA (primer IF5)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 22:

50 GCTCAGTTG TGGCGAATAA

20

(2) INFORMATION FOR SEQUENCE ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF6)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 23:

GTGGGAGCAG AAGACATTGA

20

(2) INFORMATION FOR SEQUENCE ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF7)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 24:

AATGAACAAC TTGCTGTGCT

20

(2) INFORMATION FOR SEQUENCE ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF8)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 25:

TGACAAATGT CCTCCTGGTA

20

(2) INFORMATION FOR SEQUENCE ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF9)

55

5 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 26:

AGGTAGGTAC CAGGAGGACA

20

10 (2) INFORMATION FOR SEQUENCE ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

15 (ii) MOLECULE TYPE : synthetic DNA (primer IF10)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 27:

GAGCTGCCCT CCTGGATTG

20

20 (2) INFORMATION FOR SEQUENCE ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

25 (ii) MOLECULE TYPE : synthetic DNA (primer IF11)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 28:

CAAACGTGTAT TTGCGCTCTGG

20

30 (2) INFORMATION FOR SEQUENCE ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

35 (ii) MOLECULE TYPE : synthetic DNA (primer IF12)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 29:

GTGTGAGGAG GCATTCTTCA

20

40 (2) INFORMATION FOR SEQUENCE ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 32

50

5 (B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : synthetic DNA (primer C19SF)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 30:  
10 GAATCAACTC AAAAAAGTGG AATAGATGTT AC 32

15 (2) INFORMATION FOR SEQUENCE ID NO: 31:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 32  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
20 (D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : synthetic DNA (primer C19SR)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 31:  
25 GTAACATCTA TTCCACTTTT TTGAGTTGAT TC 32

30 (2) INFORMATION FOR SEQUENCE ID NO: 32:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 30  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
35 (D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : synthetic DNA (primer C20SF)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 32:  
40 ATAGATGTTA CCCTGAGTGA GGAGGCATTC 30

45 (2) INFORMATION FOR SEQUENCE ID NO: 33:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 30  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
50 (D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : synthetic DNA (primer C20SR)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 33:

GAATGCCCTCC TCACTCAGGG TAACATCTAT

30

5 (2) INFORMATION FOR SEQUENCE ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 31
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer C21SF)

10 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 34:

15 CAAGATATTG ACCTCAGTGA AACACAGCGTG C

31

20 (2) INFORMATION FOR SEQUENCE ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 31
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer C21SR)

25 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 35:

30 GCACGCTGTT TTCACTGAGG GCAATATCTT G

31

35 (2) INFORMATION FOR SEQUENCE ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 31
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

40 (ii) MOLECULE TYPE : synthetic DNA (primer C22SF)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 36:

45 AAAACAATAA AGGCAAGCAA ACCCAGTGAC C

31

50 (2) INFORMATION FOR SEQUENCE ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 31
- (B) TYPE : nucleic acid

55

(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear

5 (ii) MOLECULE TYPE : synthetic DNA (primer C22SR)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 37:  
GGTCACTGGG TTTGCTTGCC TTTATTGTTT T 31

10 (2) INFORMATION FOR SEQUENCE ID NO: 38:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 31  
15 (B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
20 (ii) MOLECULE TYPE : synthetic DNA (primer C23SF)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 38:  
TCAGTAAAAA TAAGCAGCTT ATAACGGCC A 31

25 (2) INFORMATION FOR SEQUENCE ID NO: 39:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 31  
30 (B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
35 (ii) MOLECULE TYPE : synthetic DNA (primer C23SR)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 39:  
TGGCCAGTTA TAAGCTGCTT ATTTTTACTG A 31

40 (2) INFORMATION FOR SEQUENCE ID NO: 40:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 22  
45 (B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : synthetic DNA (primer IF 14)  
50 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 40:  
TTGGGGTTA TTGGAGGAGA TG 22

(2) INFORMATION FOR SEQUENCE ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH : 36
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

10 (ii) MOLECULE TYPE : synthetic DNA (primer DCR1F)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 41:

15 ACCACCCAGG AACCTTGCCC TGACCACTAC TACACA

36

(2) INFORMATION FOR SEQUENCE ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH : 36
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

25 (ii) MOLECULE TYPE : synthetic DNA (primer DCR1R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 42:

30 GTCAGGGCAA GGTCCTGGG TGGTCCACTT AATGGA

36

(2) INFORMATION FOR SEQUENCE ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH : 36
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

40 (ii) MOLECULE TYPE : synthetic DNA (primer DCR2F)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 43:

45 ACCGTGTGCG CCGAATGCAA GGAAGGGCGC TACCTT

36

(2) INFORMATION FOR SEQUENCE ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

- 50 (A) LENGTH : 36
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

5 (ii) MOLECULE TYPE : synthetic DNA (primer DCR2R)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 44:  
TTCCTTGCAT TCGGCGCACA CGGTCTTCCA CTTTGC 36

10 (2) INFORMATION FOR SEQUENCE ID NO: 45:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 36  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear

15 (ii) MOLECULE TYPE : synthetic DNA (primer DCR3F)  
(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 45:  
AACC CGGTGT GCAGATGTCC AGATGGGTTTC TTCTCA 36

20 (2) INFORMATION FOR SEQUENCE ID NO: 46:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 36  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear

25 (ii) MOLECULE TYPE : synthetic DNA (primer DCR3R)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 46:  
ATCTGGACAT CTGCACACGC GGTTGTGGGT GCGATT 36

30 (2) INFORMATION FOR SEQUENCE ID NO: 47:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 36  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear

35 (ii) MOLECULE TYPE : synthetic DNA (primer DCR4F)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 47:  
ACAGTTGCA AATCCGGAAA CAGTGAATCA ACTCAA 36

40 (2) INFORMATION FOR SEQUENCE ID NO: 48:  
(i) SEQUENCE CHARACTERISTICS:

45 (2) INFORMATION FOR SEQUENCE ID NO: 48:  
(i) SEQUENCE CHARACTERISTICS:

50 (2) INFORMATION FOR SEQUENCE ID NO: 48:  
(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH : 36  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : synthetic DNA (primer DCR4R)  
10 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 48:  
ACTGTTCCG GATTGCAAA CTGTATTCG CTCTGG 36

15 (2) INFORMATION FOR SEQUENCE ID NO: 49:  
(i) SEQUENCE CHARACTERISTICS:  
20 (A) LENGTH : 36  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : synthetic DNA (primer DDD1F)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 49:  
25 AATGTGGAAT AGATATTGAC CTCTGTGAAA ACAGCG 36

30 (2) INFORMATION FOR SEQUENCE ID NO: 50:  
(i) SEQUENCE CHARACTERISTICS:  
35 (A) LENGTH : 36  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : synthetic DNA (primer DDD1R)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 50:  
40 AGAGGTCAAT ATCTATTCCA CATTGGAG TTGATT 36

45 (2) INFORMATION FOR SEQUENCE ID NO: 51:  
(i) SEQUENCE CHARACTERISTICS:  
50 (A) LENGTH : 36  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : synthetic DNA (primer DDD2F)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 51:

	AGATCATCCA AGACGCACTA AAGCACTCAA AGACGT	36
5	(2) INFORMATION FOR SEQUENCE ID NO: 52:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH : 36	
10	(B) TYPE : nucleic acid	
	(C) STRANDEDNESS : single	
	(D) TOPOLOGY : linear	
	(ii) MOLECULE TYPE : synthetic DNA (primer DDD2R)	
15	(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 52:	
	GCTTAGTGC GTCTGGATG ATCTTCTTGA CTATAT	36
20	(2) INFORMATION FOR SEQUENCE ID NO: 53:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH : 29	
25	(B) TYPE : nucleic acid	
	(C) STRANDEDNESS : single	
	(D) TOPOLOGY : linear	
	(ii) MOLECULE TYPE : synthetic DNA (primer XhoI F)	
30	(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 53:	
	GGCTCGAGCG CCCAGCCGCC GCCTCCAAG	29
35	(2) INFORMATION FOR SEQUENCE ID NO: 54:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH : 20	
40	(B) TYPE : nucleic acid	
	(C) STRANDEDNESS : single	
	(D) TOPOLOGY : linear	
	(ii) MOLECULE TYPE : synthetic DNA (primer IF 16)	
45	(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 54:	
	TTTAGTGCT TTAGTGCCTG	20
50	(2) INFORMATION FOR SEQUENCE ID NO: 55:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH : 30	
	(B) TYPE : nucleic acid	

(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
5 (ii) MOLECULE TYPE : synthetic DNA (primer CL F)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 55:  
TCAGTAAAAA TAAGCTAACT GGAAATGCC 30

10 (2) INFORMATION FOR SEQUENCE ID NO: 56:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 30  
15 (B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : synthetic DNA (primer CL R)  
20 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 56:  
GGCCATTCC AGTTAGCTTA TTTTACTGA 30

25 (2) INFORMATION FOR SEQUENCE ID NO: 57:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 29  
30 (B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : synthetic DNA (primer CC R)  
35 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 57:  
CCGGATCCTC AGTGCTTAG TGCGTGCAT 29

40 (2) INFORMATION FOR SEQUENCE ID NO: 58:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 29  
45 (B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : synthetic DNA (primer CCD2 R)  
50 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 58:  
CCGGATCCTC ATTGGATGAT CTTCTTGAC 29

(2) INFORMATION FOR SEQUENCE ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH : 29
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

10 (ii) MOLECULE TYPE : synthetic DNA (primer CCD1 R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 59:

CCGGATCCTC ATATTCCACA TTTTGAGT

29

15

(2) INFORMATION FOR SEQUENCE ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH : 29
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

25 (ii) MOLECULE TYPE : synthetic DNA (primer CCR4 R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 60:

CCGGATCCTC ATTGCAAAC TGTATTCG

29

30

(2) INFORMATION FOR SEQUENCE ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH : 29
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

40 (ii) MOLECULE TYPE : synthetic DNA (primer CCR3 R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 61:

CCGGATCCTC ATTGCACAC GCGGTTGTG

29

45

(2) INFORMATION FOR SEQUENCE ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:

- 50 (A) LENGTH : 401
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

55

(ii) MOLECULE TYPE : Protein (OCIF-C19S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 62:

5	Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
	-20	-15	-10
10	Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
	-5	-1 1	5
15	Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
	10	15	20
20	Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
	25	30	35
25	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His		
	40	45	50
30	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
	55	60	65
35	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
	70	75	80
40	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
	85	90	95
45	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
	100	105	110
50	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
	115	120	125
55	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
	130	135	140
60	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
	145	150	155
65	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Ser		
	160	165	170
70	Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
	175	180	185
75	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
	190	195	200
80	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
	205	210	215
85	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
	220	225	230

Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile  
235 240 245  
5 Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile  
250 255 260  
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu  
10 265 270 275  
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr  
280 285 290  
Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser  
15 295 300 305  
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu  
310 315 320  
20 Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr  
325 330 335  
Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe  
340 345 350  
25 Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly  
355 360 365  
Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu  
30 370 375 380

(2) INFORMATION FOR SEQUENCE ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH : 401  
(B) TYPE : amino acid  
(C) STRANDEDNESS : single  
40 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-C20S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 63:

45 Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser  
-20 -15 -10  
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
40 -5 -1 1 5  
50 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro  
10 15 20  
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr

25	30	35
5	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His	
40	45	50
10	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu	
55	60	65
15	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys	
70	75	80
20	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys	
85	90	95
25	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr	
100	105	110
30	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe	
115	120	125
35	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn	
130	135	140
40	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr	
145	150	155
45	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys	
160	165	170
50	Gly Ile Asp Val Thr Leu Ser Glu Glu Ala Phe Phe Arg Phe Ala	
175	180	185
55	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp	
190	195	200
60	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile	
205	210	215
65	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys	
220	225	230
70	Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Ile	
235	240	245
75	Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile	
250	255	260
80	Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu	
265	270	275
85	Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr	
280	285	290
90	Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Ser	

295	300	305
5	Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu	
310	315	320
	Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr	
325	330	335
10	Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe	
340	345	350
	Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly	
355	360	365
15	Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu	
370	375	380

20 (2) INFORMATION FOR SEQUENCE ID NO: 64:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 401
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-C21S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 64:

30	Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser			
	-20	-15	-10	
	Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His			
35	-5	-1	1	5
	Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro			
	10	15	20	
40	Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr			
	25	30	35	
	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His			
	40	45	50	
45	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu			
	55	60	65	
	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys			
	70	75	80	
50	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys			
	85	90	95	

	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr	
100		105
5	110	
	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe	
115		120
	125	
	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn	
130		135
10	140	
	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr	
145		150
	155	
	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys	
160		165
15	170	
	Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala	
175		180
	185	
	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp	
190		195
20	200	
	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile	
205		210
	215	
25	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys	
220		225
	230	
	Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile	
235		240
30	245	
	Ile Gln Asp Ile Asp Leu Ser Glu Asn Ser Val Gln Arg His Ile	
250		255
	260	
	Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu	
265		270
35	275	
	Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr	
280		285
	290	
	Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Ser	
295		300
40	305	
	Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu	
310		315
	320	
	Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr	
325		330
45	335	
	Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe	
340		345
	350	
	Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly	
50		355
	360	365



160	165	170
5	Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala	
175	180	185
	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp	
190	195	200
10	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile	
205	210	215
	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys	
220	225	230
15	Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile	
235	240	245
	Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile	
250	255	260
20	Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu	
265	270	275
	Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr	
280	285	290
25	Ile Lys Ala Ser Lys Pro Ser Asp Gln Ile Leu Lys Leu Ser	
295	300	305
	Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu	
310	315	320
30	325	335
	Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr	
325	330	335
	Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe	
340	345	350
35	370	375
	Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly	
355	360	365
40	Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu	
	370	375
		380

45 (2) INFORMATION FOR SEQUENCE ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 401
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-C23S)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 66:  
5 Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser  
-20 -15 -10  
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
10 -5 -1 1 5  
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro  
15 10 15 20  
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr  
20 25 30 35  
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Asp Ser Trp His  
25 40 45 50  
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu  
30 55 60 65  
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys  
35 70 75 80  
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys  
40 85 90 95  
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr  
45 100 105 110  
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe  
50 115 120 125  
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn  
55 130 135 140  
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr  
60 145 150 155  
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys  
65 160 165 170  
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala  
70 175 180 185  
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp  
75 190 195 200  
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile  
80 205 210 215  
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys  
85 220 225 230

5	Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile
	235 240 245
	Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile
	250 255 260
10	Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu
	265 270 275
	Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr
	280 285 290
15	Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser
	295 300 305
	Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu
	310 315 320
20	Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr
	325 330 335
	Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe
	340 345 350
25	Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly
	355 360 365
	Asn Gln Val Gln Ser Val Lys Ile Ser Ser Leu
30	370 375 380

(2) INFORMATION FOR SEQUENCE ID NO: 67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 360
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-DCR1)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 67:

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20							-15					-10		
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr
-5							-1	1				5		
Asp	Ser	Trp	His	Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val
10												20		
Cys	Lys	Glu	Leu	Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His

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	25	30	35
5	Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu		
	40	45	50
	Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val		
10	55	60	65
	Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro		
	70	75	80
	Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg		
15	85	90	95
	Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys		
	100	105	110
	Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser		
20	115	120	125
	Thr Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe		
	130	135	140
	Phe Arg Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser		
25	145	150	155
	Val Leu Val Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser		
	160	165	170
	Val Glu Arg Ile Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe		
30	175	180	185
	Gln Leu Leu Lys Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile		
	190	195	200
	Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val		
35	205	210	215
	Gln Arg His Ile Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg		
	220	225	230
40	Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp		
	235	240	245
	Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu		
45	250	255	260
	Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr		
	265	270	275
	Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His		
50	280	285	290
	Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe		

295	300	305
Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu		
310	315	320
Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu		
325	330	335

10 (2) INFORMATION FOR SEQUENCE ID NO: 68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 359
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

15 (ii) MOLECULE TYPE : Protein (OCIF-DCR2)

20 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 68:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
-20	-15	-10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
-5	-1 1	5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
10	15	20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
25	30	35
Val Cys Ala Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe		
35	40	45
Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln		
55	60	65
Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp		
40	70	75
Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys		
85	90	95
His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly		
45	100	105
Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr		
115	115	120
Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe		
50	130	135
		140

Arg Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val  
145 150 155  
5 Leu Val Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val  
160 165 170  
Glu Arg Ile Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln  
10 175 180 185  
Leu Leu Lys Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val  
190 195 200  
Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln  
15 205 210 215  
Arg His Ile Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser  
220 225 230  
20 Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile  
235 240 245  
Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys  
250 255 260  
25 Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu  
265 270 275  
Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe  
280 285 290  
30 Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu  
295 300 305  
His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu  
35 310 315 320  
Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu  
325 330 335

40 (2) INFORMATION FOR SEQUENCE ID NO: 69:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 363

45 (B) TYPE : amino acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF-DCR3)

50 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 69:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser

	-20	-15	-10
	Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
5	-5	-1	1
	Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
	10	15	20
10	Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
	25	30	35
	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His		
	40	45	50
15	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
	55	60	65
	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
20	70	75	80
	Arg Cys Pro Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala		
	85	90	95
25	Pro Cys Arg Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu		
	100	105	110
	Thr Gln Lys Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn		
	115	120	125
30	Ser Glu Ser Thr Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu		
	130	135	140
	Glu Ala Phe Phe Arg Phe Ala Val Pro Thr Lys Phe Thr Pro Asn		
	145	150	155
35	Trp Leu Ser Val Leu Val Asp Asn Leu Pro Gly Thr Lys Val Asn		
	160	165	170
	Ala Glu Ser Val Glu Arg Ile Lys Arg Gln His Ser Ser Gln Glu		
	175	180	185
40	Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn Lys Asp		
	190	195	200
	Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu		
45	205	210	215
	Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr Phe Glu		
	220	225	230
50	Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly		
	235	240	245
	Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp		

250	255	260
5	Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp	
265	270	275
10	Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys	
280	285	290
15	Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr	
295	300	305
20	Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys	
310	315	320
325	330	335
340	Ser Cys Leu	

## (2) INFORMATION FOR SEQUENCE ID NO: 70:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 359
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF-DCR4)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 70:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser				
35	-20	-15	-10	
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His				
30	-5	-1	1	5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro				
40	10	15	20	
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr				
45	25	30	35	
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His				
50	40	45	50	
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu				
55	55	60	65	
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys				
60	70	75	80	

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Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys  
85 90 95  
5 His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr  
100 105 110  
Pro Glu Arg Asn Thr Val Cys Lys Ser Gly Asn Ser Glu Ser Thr  
115 120 125  
10 Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe  
130 135 140  
Arg Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val  
145 150 155  
15 Leu Val Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val  
160 165 170  
20 Glu Arg Ile Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln  
175 180 185  
Leu Leu Lys Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val  
190 195 200  
25 Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln  
205 210 215  
Arg His Ile Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser  
220 225 230  
30 Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile  
235 240 245  
Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys  
250 255 260  
35 Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu  
265 270 275  
Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe  
280 285 290  
40 Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu  
295 300 305  
His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu  
310 315 320  
45 Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu  
325 330 335  
50

(2) INFORMATION FOR SEQUENCE ID NO: 71:

## (i) SEQUENCE CHARACTERISTICS:

5  
 (A) LENGTH : 326  
 (B) TYPE : amino acid  
 (C) STRANDEDNESS : single  
 (D) TOPOLOGY : linear

## 10 (ii) MOLECULE TYPE : protein (OCIF-DDD1)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 71:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser  
 20 -20 -15 -10  
 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
 15 -5 -1 1 5  
 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro  
 20 10 15 20  
 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr  
 25 25 30 35  
 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His  
 30 40 45 50  
 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu  
 35 55 60 65  
 Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys  
 40 70 75 80  
 Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys  
 45 85 90 95  
 His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr  
 50 100 105 110  
 Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe  
 55 115 120 125  
 Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn  
 60 130 135 140  
 Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr  
 65 145 150 155  
 His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys  
 70 160 165 170  
 Gly Ile Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile  
 75 175 180 185  
 Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu

190	195	200
5	Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr	
205	210	215
	Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser	
220	225	230
10	Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu	
235	240	245
	Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr	
250	255	260
15	Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe	
265	270	275
	Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly	
280	285	290
20	Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu	
295	300	305

25 (2) INFORMATION FOR SEQUENCE ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 327
- (B) TYPE: amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF-DDD2)

35 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 72:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
-20	-15	-10
40	Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His	
-5	-1 1	5
	Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro	
10	15	20
45	Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr	
25	30	35
	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His	
40	45	50
50	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu	
55	60	65

Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys  
70 75 80  
5 Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys  
85 90 95  
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr  
100 105 110  
10 Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe  
115 120 125  
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn  
15 130 135 140  
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr  
145 150 155  
20 His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys  
160 165 170  
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala  
175 180 185  
25 Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp  
190 195 200  
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile  
205 210 215  
30 Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys  
220 225 230  
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile  
35 235 240 245  
Ile Gln Asp Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys  
250 255 260  
40 Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser  
265 270 275  
Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile  
280 285 290  
45 Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu  
295 300 305

50 (2) INFORMATION FOR SEQUENCE ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 399

(B) TYPE : amino acid

(C) STRANDEDNESS : single

5 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF-CL)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 73:

10	Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser	-20	-15	-10
	Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His	-5	-1 1	5
15	Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro	10	15	20
	Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr	25	30	35
20	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His	40	45	50
	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu	55	60	65
25	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys	70	75	80
	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys	85	90	95
30	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr	100	105	110
	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe	115	120	125
35	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn	130	135	140
	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr	145	150	155
40	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys	160	165	170
	Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala	175	180	185
45	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp	190	195	200
	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile			

205                    210                    215  
5 Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys  
220                    225                    230  
10 Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile  
235                    240                    245  
15 Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile  
250                    255                    260  
20 Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu  
265                    270                    275  
25 Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr  
280                    285                    290  
30 Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Ser  
295                    300                    305  
35 Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu  
310                    315                    320  
40 Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr  
325                    330                    335  
45 Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe  
340                    345                    350  
50 Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly  
355                    360                    365  
55 Asn Gln Val Gln Ser Val Lys Ile Ser  
370                    375

35 (2) INFORMATION FOR SEQUENCE ID NO: 74:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH : 351  
(B) TYPE : amino acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear

45 (ii) MOLECULE TYPE : protein (OCIF-CC)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 74:

50 Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser  
-20                    -15                    -10  
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
-5                    -1    1                    5

	Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
5	10				15							20			
	Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
	25				30							35			
10	Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
	40				45							50			
	Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
	55				60							65			
15	Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
	70				75							80			
	Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
	85				90							95			
20	His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
	100				105							110			
	Pro	Glu	Arg	Asn	Thr	Val	Cys	Lys	Arg	Cys	Pro	Asp	Gly	Phe	Phe
	115				120							125			
25	Ser	Asn	Glu	Thr	Ser	Ser	Lys	Ala	Pro	Cys	Arg	Lys	His	Thr	Asn
	130				135							140			
	Cys	Ser	Val	Phe	Gly	Leu	Leu	Leu	Thr	Gln	Lys	Gly	Asn	Ala	Thr
	145				150							155			
30	His	Asp	Asn	Ile	Cys	Ser	Gly	Asn	Ser	Glu	Ser	Thr	Gln	Lys	Cys
	160				165							170			
	Gly	Ile	Asp	Val	Thr	Leu	Cys	Glu	Glu	Ala	Phe	Phe	Arg	Phe	Ala
35	175				180							185			
	Val	Pro	Thr	Lys	Phe	Thr	Pro	Asn	Trp	Leu	Ser	Val	Leu	Val	Asp
	190				195							200			
40	Asn	Leu	Pro	Gly	Thr	Lys	Val	Asn	Ala	Glu	Ser	Val	Glu	Arg	Ile
	205				210							215			
	Lys	Arg	Gln	His	Ser	Ser	Gln	Glu	Gln	Thr	Phe	Gln	Leu	Leu	Lys
	220				225							230			
45	Leu	Trp	Lys	His	Gln	Asn	Lys	Asp	Gln	Asp	Ile	Val	Lys	Ile	
	235				240							245			
	Ile	Gln	Asp	Ile	Asp	Leu	Cys	Glu	Asn	Ser	Val	Gln	Arg	His	Ile
	250				255							260			
50	Gly	His	Ala	Asn	Leu	Thr	Phe	Glu	Gln	Leu	Arg	Ser	Leu	Met	Glu
	265				270							275			

Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr  
280 285 290  
5 Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser  
295 300 305  
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu  
310 315 320  
10 Met His Ala Leu Lys His  
325 330

(2) INFORMATION FOR SEQUENCE ID NO: 75:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 272
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

20 (ii) MOLECULE TYPE : Protein (OCIF-CDD2)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 75:

25 Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser  
-20 -15 -10  
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
-5 -1 1 5  
30 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro  
10 15 20  
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr  
35 25 30 35  
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His  
40 45 50  
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu  
45 55 60 65  
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys  
70 75 80  
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys  
85 90 95  
55 His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr  
100 105 110  
50 Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe  
115 120 125

Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn  
 130 135 140  
 5 Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr  
 145 150 155  
 His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys  
 10 160 165 170  
 Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala  
 175 180 185  
 Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp  
 15 190 195 200  
 Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile  
 205 210 215  
 20 Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys  
 220 225 230  
 Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile  
 235 240 245  
 25 Ile Gln  
 250

30 (2) INFORMATION FOR SEQUENCE ID NO: 76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 197
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-CDD1)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 76:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser  
 -20 -15 -10  
 40 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
 -5 -1 1 5  
 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro  
 10 15 20  
 45 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr  
 25 30 35  
 50 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His

5	40	45	50
	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
	55	60	65
	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
10	70	75	80
	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
	85	90	95
	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
15	100	105	110
	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
	115	120	125
	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
20	130	135	140
	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
	145	150	155
	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
25	160	165	170
	Gly Ile		
	175		

30 (a) INFORMATION FOR SEQUENCE ID NO: 77:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH : 142

(B) TYPE : *animus* and

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCT1E=CCR4)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 77:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser

-20 -15 -10

Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro

-5 -1 1 5

Asp Glu Glu Thr Ser His Gln Leu Leu Cy

10 15 20

Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Al

Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His  
40 45 50  
5 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu  
55 60 65  
10 Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys  
70 75 80  
15 Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys  
85 90 95  
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr  
100 105 110  
20 Pro Glu Arg Asn Thr Val Cys Lys  
115 120

(2) INFORMATION FOR SEQUENCE ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 106
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-CCR3)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 78:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser  
-20 -15 -10  
35 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
-5 -1 1 5  
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro  
10 15 20  
40 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr  
25 30 35  
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His  
40 45 50  
45 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu  
55 60 65  
50 Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys  
70 75 80  
Glu

85

5 (2) INFORMATION FOR SEQUENCE ID NO: 79:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 393

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-CBst)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 79:

15	Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
	-20	-15	-10
20	Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
	-5	-1	5
25	Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
	10	15	20
30	Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
	25	30	35
35	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His		
	40	45	50
40	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
	55	60	65
45	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
	70	75	80
50	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
	85	90	95
55	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
	100	105	110
60	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
	115	120	125
65	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
	130	135	140
70	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
	145	150	155
75	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
	160	165	170
80	Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		

55

175	180	185	
5	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200	
	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
10	205	210	215
	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
	220	225	230
15	Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
	235	240	245
	Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
20	250	255	260
	Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
	265	270	275
	Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
	280	285	290
25	Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
	295	300	305
	Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
	310	315	320
30	Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr		
	325	330	335
	Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe		
	340	345	350
35	Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly		
	355	360	365
	Asn Leu Val		
40	370		

## (2) INFORMATION FOR SEQUENCE ID NO: 80:

## (i) SEQUENCE CHARACTERISTICS:

45 (A) LENGTH : 321  
 (B) TYPE : amino acid  
 (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : Protein (OCIF-CSph)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 80:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser

**EP 0 816 380 A1**

	-20	-15	-10
5	Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
	-5	-1 1	5
10	Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
	15		20
15	Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
	25	30	35
20	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His		
	40	45	50
25	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
	55	60	65
30	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
	70	75	80
35	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
	85	90	95
40	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
	100	105	110
45	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
	115	120	125
50	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
	130	135	140
55	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
	145	150	155
60	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
	160	165	170
65	Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
	175	180	185
70	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
	190	195	200
75	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
	205	210	215
80	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
	220	225	230
85	Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
	235	240	245
90	Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		

250	255	260
5	Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu	
265	270	275
	Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr	
10	280	285
	Ile Lys Ala Ser Leu Asp	
	295	300

## (2) INFORMATION FOR SEQUENCE ID NO: 81:

## 15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 202
- (B) TYPE : amino acid
- (D) TOPOLOGY : linear

## 20 (ii) MOLECULE TYPE : Protein (OCIF-CBsp)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 81:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
25 -20	-15	-10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
30 -5	-1 1	5
10 15	20	
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
25 30	35	
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
35 40	45	50
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His		
55 60	65	
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
40 70	75	80
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
85 90	95	
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
45 100	105	110
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
115 120	125	
50 Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
130 135	140	

(2) INFORMATION FOR SEQUENCE ID NO: 82:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 84

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-CPst)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 82:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser  
-20 -15 -10

Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
-5 -1 1 5

Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro

10 15 20

Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr  
25 30 35

Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His

40 45 50

Thr Ser Asp Glu Cys Leu Tyr Leu Val  
55 60 63

(2) INFORMATION FOR SEQUENCE ID NO: 83:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1206

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCT16-C19S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 83:

5 ATGAAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGAGTGT 240  
 CTATACTGCA GCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 10 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 CATAGGAGCT GCCCTCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCGT 480  
 AGAAAACACA CAAATTGCAG TGTCTTGGT CTCCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
 15 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTAAA AAAGTGAAT AGATGTTACC 600  
 CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660  
 AGTGTCTTGG TAGACAATT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
 20 AACCGGCAAC ACAGCTACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900  
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960  
 25 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020  
 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080  
 GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCCCTCACA GCTTCACAAT GTACAAATTG 1140  
 TATCAGAAAGT TATTTTTAGA AATGATAGGT AACCAAGGTCC AATCAGTAAA AATAAGCTGC 1200  
 30 TTATAA 1206

## (2) INFORMATION FOR SEQUENCE ID NO: 84:

## 35 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1206
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- 40 (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : cDNA (OCIF-C20S)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 84:

45 ATGAAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGAGTGT 240  
 50 CTATACTGCA GCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300

5 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGA GCGAAATACA 420  
 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCTGT 480  
 AGAAAACACA CAAATTGCAG TGTCTTGCT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
 10 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600  
 CTGAGTGAGG AGGCATTCTT CAGGTTGCT GTTACCTACAA AGTTACGCC TAACTGGCTT 660  
 AGTGTCTTGG TAGACAATT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 15 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900  
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCAATGCAAA 960  
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAAATGG CGACCAAGAC 1020  
 20 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCAACTT TCCCAAACT 1080  
 GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCCCTCACA GCTTCACAAT GTACAAATTG 1140  
 TATCAGAAGT TATTTTTAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTGC 1200  
 TTATAA 1206

25 (2) INFORMATION FOR SEQUENCE ID NO: 85:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH : 1206  
 (B) TYPE : nucleic acid  
 (C) STRANDEDNESS : single  
 (D) TOPOLOGY : linear

35 (ii) MOLECULE TYPE : cDNA (OCIF-C21S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 85:

40 ATGAAACAAC TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCCTCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGAGTGT 240  
 45 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGA GCGAAATACA 420  
 50 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCTGT 480  
 AGAAAACACA CAAATTGCAG TGTCTTGCT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600

5 CTGTGTGAGG AGGCATTCTT CAGGTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660  
 AGTGTCTTGG TAGACAATTG GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
 AACCGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCAG TGAAAACAGC 840  
 10 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900  
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAA 960  
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCAGA TAAAAAATGG CGACCAAGAC 1020  
 ACCTTGAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080  
 GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCCCTCACA GCTTCACAAT GTACAAATTG 1140  
 15 TATCAGAAGT TATTTTTAGA AATGATAGGT ACCCAGGTCC AATCAGTAAA AATAAGCTGC 1200  
 TTATAA 1206

20 (2) INFORMATION FOR SEQUENCE ID NO: 86:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1206
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-C22S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 86:

30 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 35 TGTGACAAAT GTCCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCCAG TGACCGAGTGT 240  
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 40 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCGT 480  
 45 AGAAAACACA CAAATTGCAG TGTCTTGGT CTCCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600  
 CTGTGTGAGG AGGCATTCTT CAGGTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660  
 50 AGTGTCTTGG TAGACAATTG GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
 AACCGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900

5 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCAAGCAAA 960  
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020  
 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080  
 GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCCCTCACA GCTTCACAAT GTACAAATTG 1140  
 TATCAGAAGT TATTTTTAGA AATGATAGGT AACCAAGGTCC AATCAGTAAA AATAAGCTGC 1200  
 10 TTATAA 1206

(2) INFORMATION FOR SEQUENCE ID NO: 87:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH : 1206  
 (B) TYPE : nucleic acid  
 (C) STRANDEDNESS : single  
 20 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-C23S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 87:

25 ATGAACAACT TGCTGTGCTG CGCCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
 30 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240  
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGA GCGAAATACA 420  
 35 GTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCCTGT 480  
 AGAAAACACA CAAATTGCGAG TGTCTTGGT CTCCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600  
 40 CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660  
 AGTGTCTTGG TAGACAATT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
 45 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900  
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960  
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020  
 50 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080  
 GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCCCTCACA GCTTCACAAT GTACAAATTG 1140  
 TATCAGAAGT TATTTTTAGA AATGATAGGT AACCAAGGTCC AATCAGTAAA AATAAGCAGC 1200

TTATAA

1206

5 (2) INFORMATION FOR SEQUENCE ID NO: 88:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1083
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-DCR1)

15 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 88:

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 20 CAGGAACCTT GCCCTGACCA CTACTACACA GACAGCTGGC ACACCAGTGA CGAGTGTCTA 120  
 TACTGCAGCC CCGTGTGCAA GGAGCTGCAG TACGTCAAGC AGGAGTGCAA TCGCACCCAC 180  
 AACCGCGTGT GCGAATGCAA GGAAGGGCGC TACCTTGAGA TAGAGTTCTG CTTGAAACAT 240  
 AGGAGCTGCC CTCCCTGGATT TGGAGTGGTG CAAGCTGGAA CCCCAGAGCG AAATACAGTT 300  
 25 TGAAAAGAT GTCCAGATGG GTTCTTCTCA AATGAGACGT CATCTAAAGC ACCCTGTAGA 360  
 AAACACACAA ATTGCAGTGT CTTTGGTCTC CTGCTAACTC AGAAAGGAAA TGCAACACAC 420  
 GACAACATAT GTTCCGGAAA CAGTGAATCA ACTCAAAAAT GTGGAATAGA TGTACCCCTG 480  
 TGTGAGGGAGG CATTCTTCAG GTTTGCTGTT CCTACAAAGT TTACGCCTAA CTGGCTTAGT 540  
 30 GTCTTGGTAG ACAATTGCCC TGGCACCAAA GTAAACGCAG AGAGTGTAGA GAGGATAAAA 600  
 CGGCAACACA GCTCACAAAGA ACAGACTTTC CAGCTGCTGA AGTTATGGAA ACATCAAAC 660  
 AAAGACCAAG ATATAGTCAA GAAGATCATC CAAGATATTG ACCTCTGTGA AAACAGCGTG 720  
 35 CAGCGGCACA TTGGACATGC TAACCTCACC TTCGAGCAGC TTCGTAGCTT GATGGAAAGC 780  
 TTACCGGGAA AGAAAAGTGGG AGCAGAAGAC ATTGAAAAAA CAATAAAGGC ATGCAAACCC 840  
 AGTGACCAAGA TCCTGAAGCT GCTCAGTTG TGGCGAATAA AAAATGGCGA CCAAGACACC 900  
 40 TTGAAGGGCC TAATGCACGC ACTAAAGCAC TCAAAGACGT ACCACTTTCC CAAAAGTGT 960  
 ACTCAGAGTC TAAAGAAGAC CATCAGGTT CTTCACAGCT TCACAATGTA CAAATTGTAT 1020  
 CAGAAGTTAT TTTAGAAT GATAGGTAAC CAGGTCCAAT CAGTAAAAAT AAGCTGCTTA 1080  
 TAA 1083

45 (2) INFORMATION FOR SEQUENCE ID NO: 89:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1080
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single

55

(D) TOPOLOGY : linear

5 (ii) MOLECULE TYPE : cDNA (OCIF-DCR2)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 89:

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCTCTGG TACCTACCTA AAACAACACT GTACAGCAA GTGGAAGACC 180  
GTGTGCGCCG AATGCAAGGA AGGGCGCTAC CTTGAGATAG AGTTCTGCTT GAAACATAGG 240  
AGCTGCCCTC CTGGATTGG AGTGGTGCAA GCTGGAACCC CAGAGCGAAA TACAGTTGC 300  
AAAAGATGTC CAGATGGTT CTTCTCAAAT GAGACGTAT CTAAAGCACC CTGTAGAAAA 360  
CACACAAATT GCAGTGTCTT TGGTCTCCTG CTAACTCAGA AAGGAAATGC AACACACGAC 420  
AACATATGTT CCGGAAACAG TGAATCAACT CAAAAATGTG GAATAGATGT TACCCCTGTGT 480  
GAGGAGGCAT TCTTCAGGTT TGCTGTTCTT ACAAAAGTTA CGCCTAACTG GCTTAGTGTC 540  
TTGGTAGACA ATTTGCCTGG CACCAAAGTA AACGCAGAGA GTGTAGAGAG GATAAAACGG 600  
CAACACAGCT CACAAGAACAA GACTTCCAG CTGCTGAAGT TATGGAACAA TCAAAACAAA 660  
GACCAAGATA TAGTCAAGAA GATCATCCAA GATATTGACC TCTGTAAAA CAGCGTGCAG 720  
CGGCACATTG GACATGCTAA CCTCACCTTC GAGCAGCTTC GTAGCTTGAT GGAAAGCTTA 780  
CCGGGAAAGA AAGTGGGAGC AGAAGACATT GAAAAAAACAA TAAAGGCATG CAAACCCAGT 840  
GACCAGATCC TGAAGCTGCT CAGTTGTGG CGAATAAAAAA ATGGCGACCA AGACACCTTG 900  
AAGGGCCTAA TGCACGCCTA AAAGCACTCA AAGACGTACC ACTTTCCAA AACTGTCACT 960  
CAGAGTCTAA AGAAGACCAT CAGGTTCCCTT CACAGCTTCA CAATGTACAA ATTGTATCAG 1020  
AAGTTATTT TAGAAATGAT AGGTAACCAG GTCCAATCAG TAAAAATAAG CTGCTTATAA 1080

35 (2) INFORMATION FOR SEQUENCE ID NO: 90:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1092

40 (B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-DCR3)

45 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 90:

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCTCTGG TACCTACCTA AAACAACACT GTACAGCAA GTGGAAGACC 180  
GTGTGCGCCG CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCCAG TGACGAGTGT 240

5 CTATACTGCA GCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 CACAACCGCG TGTGCAGATG TCCAGATGGG TTCTCTCAA ATGAGACGTC ATCTAAAGCA 360  
 CCCTGTAGAA AACACACAAA TTGCAGTGTG TTTGGTCTCC TGCTAACTCA GAAAGGAAAT 420  
 GCAACACACG ACAACATATG TTCCGGAAAC AGTGAATCAA CTCAAAAATG TGGAATAGAT 480  
 GTTACCCGT GTGAGGAGGC ATTCTTCAGG TTTGCTGTTC CTACAAAGTT TACGCCAAC 540  
 10 TGGCTTAGTG TCTTGGTAGA CAATTGCTT GGCACCAAAG TAAACGCAGA GAGTGTAGAG 600  
 AGGATAAAAC GGCAACACAG CTCACAAGAA CAGACTTCC AGCTGCTGAA GTTATGGAAA 660  
 CATCAAAACA AAGACCAAGA TATAGTCAAG AAGATCATCC AAGATATTGA CCTCTGTGAA 720  
 15 AACAGCGTGC AGCGGCACAT TGGACATGCT AACCTCACCT TCGAGCAGCT TCGTAGCTTG 780  
 ATGGAAAGCT TACCGGGAAA GAAAGTGGGA GCAGAAGACA TTGAAAAAAC AATAAAGGCA 840  
 TGCAAACCCA GTGACCAAGAT CCTGAAGCTG CTCAGTTGT GGCGAATAAA AAATGGCGAC 900  
 CAAGACACCT TGAAGGGCCT AATGCACGCA CTAAAGCACT CAAAGACGTA CCACTTCCC 960  
 20 AAAACTGTCA CTCAGAGTCT AAAGAAGACC ATCAGGTTCC TTCACAGCTT CACAATGTAC 1020  
 AAATTGTATC AGAAGTTATT TTTAGAAATG ATAGGTAACC AGGTCCAATC AGTAAAATA 1080  
 AGCTGCTTAT AA 1092

## 25 (2) INFORMATION FOR SEQUENCE ID NO: 91:

## (i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH : 1080  
 (B) TYPE : nucleic acid  
 (C) STRANDEDNESS : single  
 (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : cDNA (OCIF-DCR4)

## 35 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 91:

40 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCCAG TGACCGAGTGT 240  
 CTATACTGCA GCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 45 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTGAAA 360  
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
 GTTGTGAAAT CCGGAAACAG TGAATCAACT CAAAAATGTG GAATAGATGT TACCCGTGTT 480  
 50 GAGGAGGCAT TCTTCAGGTT TGCTGTTCCCT ACAAAAGTTA CGCCTAACTG GCTTAGTGTC 540  
 TTGGTAGACA ATTGCGCTGG CACCAAAGTA AACGCAGAGA GTGTAGAGAG GATAAAACGG 600  
 CAACACAGCT CACAAGAACAA GACTTTCCAG CTGCTGAAGT TATGGAAACA TCAAAACAAA 660

5 GACCAAGATA TAGTCAAGAA GATCATCAA GATATTGACC TCTGTAAAAA CAGCGTGCAG 720  
 CGGCACATTG GACATGCTAA CCTCACCTTC GAGCAGCTTC GTAGCTTGAT GGAAAGCTTA 780  
 10 CCGGGAAAGA AAGTGGGAGC AGAAGACATT GAAAAAACAA TAAAGGCATG CAAACCCAGT 840  
 GACCAGATCC TGAAGCTGCT CAGTTGTGG CGAATAAAAAA ATGGCGACCA AGACACCTG 900  
 AAGGGCTAA TGACACGCACT AAAGCACTCA AAGACGTACC ACTTTCCCAA AACTGTCACT 960  
 15 CAGAGTCTAA AGAAGACCAT CAGGTTCCCTT CACAGCTTCA CAATGTACAA ATTGTATCAG 1020  
 AAGTTATTT TAGAAATGAT AGGTAACCAG GTCCAATCAG TAAAAATAAG CTGCTTATAA 1080

## (2) INFORMATION FOR SEQUENCE ID NO: 92:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 981
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-DDD1)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 92:

25 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 30 TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGAGTGT 240  
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATGCACC 300  
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 35 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
 GTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCCTGT 480  
 AGAAAACACA CAAATTGCAG TGTCTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
 40 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATATTGAC 600  
 CTCTGTGAAA ACAGCGTGCA GCGGCACATT GGACATGCTA ACCTCACCTT CGAGCAGCTT 660  
 CGTAGCTTGA TGGAAAGCTT ACCGGGAAAG AAAGTGGGAG CAGAAGACAT TGAAAAAAACA 720  
 45 ATAAAGGCAT GCAAACCCAG TGACCAAGATC CTGAAGCTGC TCAGTTGTG GCGAATAAAA 780  
 AATGGCGACC AAGACACCTT GAAGGGCCTA ATGCACGCAC TAAAGCACTC AAAGACGTAC 840  
 CACTTTCCCA AAACTGTCAC TCAGAGTCTA AAGAAGACCA TCAGGTTCCCT TCACAGCTTC 900  
 ACAATGTACA AATTGTATCA GAAGTTATTT TTAGAAATGA TAGGTAACCA GGTCCAATCA 960  
 50 GTAAAAATAA GCTGCTTATA A 981

## (2) INFORMATION FOR SEQUENCE ID NO: 93:

## (i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH : 984  
 (B) TYPE : nucleic acid  
 (C) STRANDEDNESS : single  
 (D) TOPOLOGY : linear

## 10 (ii) MOLECULE TYPE : cDNA (OCIF-DDD2)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 93:

15 ATGAAACAAC TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240  
 20 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCCTGT 480  
 25 AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600  
 CTGTGTGAGG AGGCATTCTT CAGGTTGCT GTTCCCTACAA AGTTTACGCC TAACTGGCTT 660  
 AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
 30 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGACG CACTAAAGCA CTCAAAGACG 840  
 TACCACTTTC CCAAAACTGT CACTCAGAGT CTAAAGAAGA CCATCAGGTT CCTTCACAGC 900  
 35 TTCACAATGT ACAAAATTGTA TCAGAAGTTA TTTTAGAAA TGATAGGTAA CCAGGTCCAA 960  
 TCAGTAAAAA TAAGCTGCTT ATAA 984

## 40 (2) INFORMATION FOR SEQUENCE ID NO: 94:

## (i) SEQUENCE CHARACTERISTICS:

45 (A) LENGTH : 1200  
 (B) TYPE : nucleic acid  
 (C) STRANDEDNESS : single  
 (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : cDNA (OCIF-CL)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 94:

50 ATGAAACAAC TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60

5 CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGAGTGT 240  
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 10 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGA GCGAAATACA 420  
 GTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCGT 480  
 AGAAAACACA CAAATTGCAG TGTCTTGTT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
 CACGACAACA TATGTTCCGG AAACAGTGA TCAACTCAA AATGTGGAAT AGATGTTACC 600  
 15 CTGTTGAGG AGGCATTCTT CAGGTTTGCT GTTACCTACAA AGTTTACGCC TAACTGGCTT 660  
 AGTGTCTTGG TAGACAATT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
 AAACGGCAAC ACAGCTACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 20 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900  
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAA 960  
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020  
 25 ACCTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCAACTT TCCAAAACT 1080  
 GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCTTCACA GCTTCACAAT GTACAAATTG 1140  
 TATCAGAAGT TATTTTTAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTAA 1200

30 (2) INFORMATION FOR SEQUENCE ID NO: 95:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1056
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

35 (ii) MOLECULE TYPE : cDNA (OCIF-CC)

40 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 95:

45 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGAGTGT 240  
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 50 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGA GCGAAATACA 420

5 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCGT 480  
 AGAAAACACA CAAATTGCAG TGTCTTGGT CTCCGTCAA CTCAGAAAGG AAATGCAACA 540  
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600  
 CTGTGTGAGG AGGCATTCTT CAGGTTGCT GTTCTACAA AGTTTACGCC TAACTGGCTT 660  
 AGTGTCTTGG TAGACAATT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
 10 AACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900  
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAA 960  
 15 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAAATGG CGACCAAGAC 1020  
 ACCTTGAGG GCCTAATGCA CGCACTAAAG CACTGA 1056

20 (2) INFORMATION FOR SEQUENCE ID NO: 96:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 819
- (B) TYPE : nucleic acid
- 25 (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CDD2)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 96:

30 ATGAACAAC TGCCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAA GTGGAAGACC 180  
 35 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240  
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 40 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCGT 480  
 AGAAAACACA CAAATTGCAG TGTCTTGGT CTCCGTCAA CTCAGAAAGG AAATGCAACA 540  
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600  
 45 CTGTGTGAGG AGGCATTCTT CAGGTTGCT GTTCTACAA AGTTTACGCC TAACTGGCTT 660  
 AGTGTCTTGG TAGACAATT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 50 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAATGA 819

(2) INFORMATION FOR SEQUENCE ID NO: 97:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 594
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

10 (ii) MOLECULE TYPE : cDNA (OCIF-CDD1)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 97:

15 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240  
20 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
CATAGGAGCT GCCCTCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
25 GTTGCAAAA GATGTCCAGA TGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCTGT 480  
AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
CACGACAACA TATGTTCCGG AACAGTGAA TCAACTCAA AATGTGGAAT ATGA. 594

30 (2) INFORMATION FOR SEQUENCE ID NO: 98:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 432
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

35 (ii) MOLECULE TYPE : cDNA (OCIF-CCR4)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 98:

40 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240  
45 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
CATAGGAGCT GCCCTCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
50 GTTTGCAAAT GA 432

(2) INFORMATION FOR SEQUENCE ID NO: 99:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH : 321
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

10 (ii) MOLECULE TYPE : cDNA (OCIF-CCR3)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 99:

15 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
20 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCCAG TGACGAGTGT 240  
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
CACAACCGCG TGTGCGAATG A 321

25 (2) INFORMATION FOR SEQUENCE ID NO: 100:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH : 1182
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

35 (ii) MOLECULE TYPE : cDNA (OCIF-CBst)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 100:

40 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCCAG TGACGAGTGT 240  
45 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGA GCGAAATACA 420  
50 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCCTGT 480  
AGAAAACACA CAAATTGCAG TGTCTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600  
CTGTGTGAGG AGGCATTCTT CAGGTTGCT GTTCCCTACAA AGTTTACGCC TAACTGGCTT 660

5 AGTGTCTTGG TAGACAATT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
 AACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
 10 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900  
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCAATGCAA 960  
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020  
 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080  
 GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCCTTCACA GCTTCACAAT GTACAAATTG 1140  
 TATCAGAAGT TATTTTTAGA AATGATAGGT AACCTAGTCT AG 1182

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(2) INFORMATION FOR SEQUENCE ID NO: 101:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH : 966  
 (B) TYPE : nucleic acid  
 (C) STRANDEDNESS : single  
 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CSph)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 101:

30 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCCAG TGACGAGTGT 240  
 35 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCCTGT 480  
 40 AGAAAACACA CAAATTGAG TGTCTTTGGT CTCCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600  
 CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCCTACAA AGTTACGCC TAACTGGCTT 660  
 45 AGTGTCTTGG TAGACAATT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
 50 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900  
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCTAGTCTA 960  
 GACTAG 966

55

(2) INFORMATION FOR SEQUENCE ID NO: 102:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 564
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CBsp)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 102:

15 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
20 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGAGTGT 240  
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
CACAAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
25 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCCTGT 480  
AGAAAAACACA CAAATTGCAG TGTCTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
30 CACGACAAACA TATGTTCCGG CTAG 564

(2) INFORMATION FOR SEQUENCE ID NO: 103:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 255
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-Pst)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 103:

45 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
50 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGAGTGT 240  
CTATACCTAG TCTAG 255

(2) INFORMATION FOR SEQUENCE ID NO: 104:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH : 1317  
 (B) TYPE : nucleic acid  
 (C) STRANDEDNESS : double  
 10 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : human OCIF genomic DNA-1

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 104:

15	CTGGAGACAT ATAACTTGAA CACTTGGCCC TGATGGGAA GCAGCTCTGC AGGGACTTT	60
	TCAGCCATCT GTAAACAATT TCAGTGGCAA CCCGCGAACT GTAATCCATG AATGGGACCA	120
	CACTTTACAA GTCATCAAGT CTAACCTCTA GACCAGGGAA TTAATGGGG AGACAGCGAA	180
20	CCCTAGAGCA AAGTGCCAAA CTTCTGTCGA TAGCTTGAGG CTAGTGGAAA GACCTCGAGG	240
	AGGCTACTCC AGAAGTTCAG CGCGTAGGAA GCTCCGATAC CAATAGCCCT TTGATGATGG	300
	TGGGGTTGGT GAAGGGAACA GTGCTCCGCA AGGTTATCCC TGCCCCAGGC AGTCCAATT	360
25	TCACTCTGCA GATTCTCTCT GGCTCTAACT ACCCCAGATA ACAAGGAGTG AATGCAGAAT	420
	AGCACGGGCT TTAGGGCCAA TCAGACATTA GTAGAAAAAA TTCTCTACTAC ATGGTTTATG	480
	TAAACTTGAA GATGAATGAT TCGGAACCTCC CCGAAAAGGG CTCAGACAAT GCCATGCATA	540
	AAGAGGGGCC CTGTAATTG AGGTTTCAGA ACCCGAAGTG AAGGGGTCAAG GCAGCCGGGT	600
30	ACGGCGGAAA CTCACAGCTT TCGCCCGAGCG AGAGGACAAA GGTCTGGAC ACACCTCAAC	660
	TGCGTCCGGA TCTTGGCTGG ATCGGACTCT CAGGGTGGAG GAGACACAAG CACAGCAGCT	720
	GCCCAGCGTG TGCCCAGCCC TCCCACCGCT GGTCCCGGCT GCCAGGAGGC TGGCCGCTGG	780
	CGGGAAGGGG CCGGGAAACC TCAGAGCCCC GCGGAGACAG CAGCCGCCCT GTTCCCTCAGC	840
35	CCGGTGGCTT TTTTTTCCCC TGCTCTCCCA GGGGACAGAC ACCACCGCCC CACCCCTCAC	900
	GCCCCACCTC CCTGGGGGAT CCTTTCGGCC CCAGCCCTGA AAGCGTTAAT CCTGGAGCTT	960
	TCTGCACACC CCCCAGCCGC TCCCGCCCAA GCTTCCTAAA AAAGAAAGGT GCAAAGTTG	1020
40	GTCCAGGATA GAAAAATGAC TGATCAAAGG CAGGCGATAC TTCTGTTGC CGGGACGCTA	1080
	TATATAACGT GATGAGCGCA CGGGCTGCGG AGACGCACCG GAGCGCTCGC CCAGCCGCCG	1140
	CCTCCAAGCC CCTGAGGTTT CGGGGGACCA CA ATG AAC AAG TTG CTG TGC TGC	1193

Met Asn Lys Leu Leu Cys Cys

45 -20 -15

45	GCG CTC GTG GTAAAGTCCCT GGGCCAGCCG ACGGGTGCCG GGCCTGGGG	1242
	Ala Leu Val	

50	GAGGCTGCTG CCACCTGGTC TCCCAACCTC CCAGCGGACC GGCGGGAAA AAGGCTCCAC	1302
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TCGCTCCCTC CCAAG

1317

5 (2) INFORMATION FOR SEQUENCE ID NO: 105:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH :

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : human OCIF genomic DNA-2

15 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 105:

GCTTACTTTG TGCCAAATCT CATTAGGCTT AAGGTAATAC AGGACTTTGA GTCAAATGAT @60

20 ACTGTTGCAC ATAAGAACAA ACCTATTTTC ATGCTAAGAT GATGCCACTG TGTTCCCTTC 120

TCCTTCTAG TTT CTG GAC ATC TCC ATT AAG TGG ACC ACC CAG GAA ACG TTT 171

Phe Leu Asp Ile Ser Ile Lys Trp Thr Thr Gln Glu Thr Phe

-10 -5 -1 1

25

CCT CCA AAG TAC CTT CAT TAT GAC GAA GAA ACC TCT CAT CAG CTG TTG 219

Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His Gln Leu Leu

30

5 10 15

35 TGT GAC AAA TGT CCT CCT GGT ACC TAC CTA AAA CAA CAC TGT ACA GCA 267

Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala

40

20 25 30 35

45 AAG TGG AAG ACC GTG TGC GCC CCT TGC CCT GAC CAC TAC TAC ACA GAC 315

Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp

50

40 45 50

55 AGC TGG CAC ACC AGT GAC GAG TGT CTA TAC TGC AGC CCC GTG TGC AAG 363

Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys

60

55 60 65

65

GAG CTG CAG TAC GTC AAG CAG GAG TGC AAT CGC ACC CAC AAC CGC GTG 411

Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val

70

70 75 80

55

**EP 0 816 380 A1**

5	TGC GAA TGC AAG GAA GGG CGC TAC CTT GAG ATA GAG TTC TGC TTG AAA Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys 85	90	95	459
10	CAT AGG AGC TGC CCT CCT GGA TTT GGA GTG GTG CAA GCT G GTACGTGTCA His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala 100	105	110	509
15	ATGTGCAGCA AAATTAATTA GGATCATGCA AAGTCAGATA GTTGTGACAG TTTAGGAGAA CACTTTGTT CTGATGACAT TATAGGATAG CAAATTGCAA AGGTAATGAA ACCTGCCAGG TAGGTACTAT GTGTCTGGAG TGCTTCCAAA GGACCATTGC TCAGAGGAAT ACTTGCCAC TACAGGGCAA TTTAATGACA AATCTCAAAT GCAGCAAATT ATTCTCTCAT GAGATGCATG ATGGTTTTTT TTTTTTTTT TAAAGAAACA AACTCAAGTT GCACTATTGA TAGTTGATCT 20	749	689	569
25	ATACCTCTAT ATTTCACTTC AGCATGGACA CCTTCAAACACT GCAGCACTTT TTGACAAACA TCAGAAATGT TAATTTATAC CAAGAGAGTA ATTATGCTCA TATTAATGAG ACTCTGGAGT GCTAACAAATA AGCAGTTATA ATTAATTATG TAAAAAAATGA GAATGGTGAG GGGATTGCA TTTCATTATT AAAAACAAAGG CTAGTTCTTC CTTAGCATG GGAGCTGAGT GTTTGGGAGG GTAAGGACTA TAGCAGAACATC TCTTCAATGA GCTTATTCTT TATCTTAGAC AAAACAGATT 30	989	869	629
35	GTCAAGCCAA GAGCAAGCAC TTGCCTATAA ACCAAGTGCCT TTCTCTTTG CATTGAAAC AGCATTGGTC AGGGCTCATG TGTATTGAAT CTTTAAACC AGTAACCCAC GTTTTTTTTC TGCCACATTT GCGAAGCTTC AGTGCAGCCT ATAACCTTTTC ATAGCTTGAG AAAATTAAGA GTATCCACTT ACTTAGATGG AAGAAGTAAT CAGTATAGAT TCTGATGACT CAGTTGAAG 40	1229	1169	749
45	CAGTGTCTCT CAACTGAAGC CCTGCTGATA TTTAAGAAA TATCTGGATT CCTAGGCTGG ACTCCTTTT GTGGGCAGCT GTCCTGCGCA TTGTAGAATT TTGGCAGCAC CCCTGGACTC TAGCCACTAG ATACCAATAG CAGCCTTCC CCCATGTGAC AGCCAAAAT GTCTTCAGAC ACTGTCAAAT GTCGCCAGGT GGCAAAATCA CTCCTGGTTG AGAACAGGGT CATCAATGCT AAGTATCTGT AACTATTTTA ACTCTCAAAA CTTGTGATAT ACAAAAGTCTA AATTATTAGA CGACCAATAC TTAGGTTTA AAGGCATACA AATGAAACAT TCAAAAATCA AAATCTATTG TGTTTCTCAA ATAGTGAATC TTATAAAATT AATCACAGAA GATGCAAATT GCATCAGAGT 50	1409	1349	1289
55	CCCTTAAAT TCCTCTTCGT ATGAGTATTG GAGGGAGGAA TTGGTGTAG TTCCTACTTT CTATTGGATG GTACTTTGAG ACTCAAAAGC TAAGCTAAGT TGTGTGTGTCAGGGTGC GGGTGTGGAA TCCCCTCAGA TAAAAGCAAA TCCATGTAAT TCATTCAAGTA AGTTGTATAT GTAGAAAAAT GAAAAGTGGG CTATGCAGCT TGAAACTAG AGAATTGAA AAAATAATGG AAATCACAAG GATCTTTCTT AAATAAGTAA GAAAATCTGT TTGTAGAATG AAGCAAGCAG GCAGCCAGAA GACTCAGAAC AAAAGTACAC ATTTACTCT GTGTACACTG GCAGCACAGT GGGATTATT TACCTCTCCC TCCCTAAAAA CCCACACAGC GGTTCTCTT GGGAAATAAG	1829	1769	1589
				2189

5	AGGTTTCCAG CCCAAAGAGA AGGAAAGACT ATGTGGTGT ACTCTAAAAA GTATTTAATA ACCGTTTGT TGTTGCTGTT GCTGTTTGA AATCAGATTG TCTCCTCTCC ATATTTTATT TACTTCATTC TGTAAATTCC TGTGGAATTAA CTTAGAGCAA GCATGGTGA TTCTCAACTG TAAAGCCAAA TTTCTCCATC ATTATAATT CACATTTGC CTGGCAGGTT ATAATTTTA TATTTCCACT GATAGTAATA AGGTAAAATC ATTACTAGA TGGATAGATC TTTTCATAA AAAGTACCAT CAGTTATAGA GGGAGTCAT GTTCATGTT AGGAAGGTCA TTAGATAAAG CTTCTGAATA TATTATGAAA CATTAGTTCT GTCATTCTTA GATTCTTTT GTAAATAAAC TTTAAAAGCT AACTTACCTA AAAGAAATAT CTGACACATA TGAACCTCTC ATAGGATGC AGGAGAAAGAC CCAAGCCACA GATATGTATC TGAAGAATGA ACAAGATTCT TAGGCCCGC 10 ACGGTGGCTC ACATCTGTA TCTCAAGAGT TTGAGAGGTC AAGGCGGGCA GATCACCTGA GGTCAGGAGT TCAAGACCAAG CCTGGCCAAC ATGATGAAAC CCTGCCTCTA CTAAAATAC AAAAATTAGC AGGGCATGGT GGTGCATGCC TGCAACCCCTA GCTACTCAGG AGGCTGAGAC AGGAGAAATCT CTTGAACCCCT CGAGGCGGAG GTTGTGGTGA GCTGAGATCC CTCTACTGCA 20 CTCCAGCCTG GGTGACAGAG ATGAGACTCC GTCCCTGCCG CCGCCCCCGC CTTCCCCCCC AAAAAGATTC TTCTTCATGC AGAACATACG GCAGTCACA AAGGGAGACC TGGGTCCAGG TGTCCAAGTC ACTTATTTCG AGTAAATTAG CAATGAAAGA ATGCCATGGA ATCCCTGCC AAATACCTCT GCTTATGATA TTGTTAGAATT TGATATAGAG TTGTATCCCA TTTAAGGAGT 25 AGGATGTAGT AGGAAAGTAC TAAAAACAAA CACACAAACA GAAAACCCCTC TTTGCTTTGT AAGGTGGITC CTAAGATAAT GTCAGTGCAA TGCTGAAAT AATATTTAAT ATGTGAAGGT TTTAGGCTGT GTTTTCCCT CCTGTTCTT TTTCTGCCA GCCCTTGTC ATTTTGCAG GTCAATGAAT CATGTAGAAA GAGACAGGAG ATGAAACTAG AACCAAGTCCA TTTGCCCC 30 TTTTTATTT TCTGGTTTG GTAAAAGATA CAATGAGGTA GGAGGTTGAG ATTTATAAAT GAAGTTTAAT AAGTTCTGT AGCTTGTATT TTTCTTTTC ATATTGTTA TCTTGCATAA GCCAGAATTG GCCTGTAAAA TCTACATATG GATATTGAAG TCTAAATCTG TTCAACTAGC TTACACTAGA TGGAGATATT TTCATATTCA GATACACTGG AATGTATGAT CTAGCCATGC GTAATATAGT CAAGTGTGTT AAGGTATTAA TTTTAATAG CGCTTTAGT TGTGGACTGG 35 TTCAAGTTTT TCTGCCAATG ATTTCTTCAA ATTTATCAAAT TATTTTCCA TCATGAAGTA AAATGCCCTT GCAGTCACCC TTCTGAAGT TTGAACGACT CTGCTGTTT AAACAGTTA AGCAAATGGT ATATCATCTT CCGTTTACTA TGTAGCTAA CTGCAGGCTT ACGCTTTGA GTCAGCGGCC AACTTTATTG CCACCTTCAA AAGTTTATTA TAATGTTGTA AATTTTACT 40 TCTCAAGTT AGCATACTTA GGAGTTGCTT CACAATTAGG ATTCAAGGAAA GAAAGAACTT CAGTAGGAAC TGATTGGAAT TTAATGATGC AGCATTCAAT GGGTACTAAT TTCAAAGAAT GATATTACAG CAGACACACA GCAGTTATCT TGATTTCTA GGAATAATTG TATGAAGAAT ATGGCTGACA ACACGGCCTT ACTGCCACTC AGCGGAGGCT GGACTAATGA ACACCCCTACC 50 CTTCTTCCTT TTCTCTCAC ATTTCATGAG CGTTTGTAG GTAACGAGAA AATTGACTTG CATTGCATT ACAAGGAGGA GAAACTGGCA AAGGGGATGA TGGTGGAAAGT TTTGTTCTGT 4349
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**EP 0 816 380 A1**

5	CTAATGAAGT GAAAAATGAA AATGCTAGAG TTTTGTGCAA CATAATAGTA GCACTAAAAA CCAAGTGAAA AGTCTTCCA AAACTGTGTT AAGAGGGCAT CTGCTGGAA ACGATTTGAG GAGAAGGTAC TAAATTGCTT GGTATTTCC GTAG GA ACC CCA GAG CGA AAT ACA	4409 4469 4523
	Gly Thr Pro Glu Arg Asn Thr	
	115	
10	GTT TGC AAA AGA TGT CCA GAT GGG TTC TTC TCA AAT GAG ACG TCA TCT Val Cys Lys Arg Cys Pro Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser	4571
	120 125 130 135	
15	AAA GCA CCC TGT AGA AAA CAC ACA AAT TGC AGT GTC TTT GGT CTC CTG Lys Ala Pro Cys Arg Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu	4619
	140 145 150	
20	CTA ACT CAG AAA GGA AAT GCA ACA CAC GAC AAC ATA TGT TCC GGA AAC Leu Thr Gln Lys Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn	4667
	155 160 165	
25	AGT GAA TCA ACT CAA AAA TGT GGA ATA G GTAATTACAT TCCAAAATAC Ser Glu Ser Thr Gln Lys Cys Gly Ile	4715
	170 175	
30	GTCTTGAC GATTTGTAG TATCATCTCT CTCTCTGAGT TGAACACAAG GCCTCCAGCC ACATTCTGG TCAAACCTAC ATTTCCCTT TCTTGAATCT TAACCAGCTA AGGCTACTCT	4775 4835
	CGATGCATTA CTGCTAAAGC TACCACTCAG AATCTCTCAA AAACTCATCT TCTCACAGAT	4895
35	AACACCTCAA AGCTTGATT TCTCTCCTT CACACTGAAA TCAAATCTTG CCCATAGGCA AAGGGCAGTG TCAAGTTGC CACTGAGATG AAATTAGGAG AGTCCAAACT GTAGAATTCA	4955 5015
	CGTTGTGTGT TATTACTTTC ACGAATGTCT GTATTATTAA CTAAAGTATA TATTGGCAAC	5075
40	TAAGAAGCAA AGTGTATATA ACATGATGAC AAATTAGGCC AGGCATGGTG GCTTACTCCT ATAATCCCAA CATTGGGG GGCCAGGTA GGCAAGTCAC TTGAGGTAG GATTCAAGA	5135 5195
	CCAGCCTGAC CAACATGGTG AAACCTTGTC TCTACTAAAA ATACAAAAAT TAGCTGGCA	5255
45	TGGTAGCAGG CACTTCTAGT ACCAGCTACT CAGGGCTGAG GCAGGAGAAT CGCTTGAACC CAGGAGATGG AGGTTGCAGT GAGCTGAGAT TGTACCACTG CACTCCAGTC TGGCAACAG	5315 5375
	AGCAAGATTT CATCACACAC ACACACACAC ACACACACAC ACACATTAGA AATGTGTACT	5435
50	TGGCTTGTT ACCTATGGTA TTAGTCATC TATTGCATGG AACTTCCAAG CTACTCTGGT TGTGTTAAGC TCTTCATTGG GTACAGGTCA CTAGTATTAA GTTCAGGTAA TTGGATGCA	5495 5555

5	TTCCACGGTA GTGATGACAA TTCATCAGGC TAGTGTGTGT GTTCACCTTG TCACTCCCAC CACTAGACTA ATCTCAGACC TTCACTAAA GACACATTAC ACTAAAGATG ATTTGCTTT	5615 5675
10	TTGTGTTAA TCAAGCAATG GTATAAACCA GCTTGAECTCT CCCCAAACAG TTTTCGTAC TACAAAGAAG TTTATGAAGC AGAGAAATGT GAATTGATAT ATATATGAGA TTCTAACCCA GTTCCAGCAT TGTTTCATTG TGTAATTGAA ATCATAGACA AGCCATTAA GCCTTGCTT TCTTATCTAA AAAAAAAA AAAAAAATGA AGGAAGGGGT ATTAAAAGGA GTGATCAAAT	5735 5795 5855 5915
15	TTAACATTC TCTTAATTA ATTCATTTT AATTTACTT TTTTCATT ATTGTGCACT TACTATGTGG TACTGTGCTA TAGAGGTTT AACATTATA AAAACACTGT GAAAGTTGCT TCAGATGAAT ATAGGTAGTA GAACGGCAGA ACTAGTATT AAAGCCAGGT CTGATGAATC CAAAAACAAA CACCCATTAC TCCCATTTC TGGGACATAC TTACTCTACC CAGATGCTCT	5975 6035 6095 6155
20	GGGCTTGTA ATGCCTATGT AAATAACATA GTTTATGTT TGGTTATTTT CCTATGTAAT GTCTACTTAT ATATCTGTAT CTATCTCTG CTTTGTTC AAAGGTAAC TATGTGCTA AATGTGGCA AAAAATAACA CACTATTCCA AATTACTGTT CAAATTCCCT TAAGTCAGTG ATAATTATTT GTTTGACAT TAATCATGAA GTTCCCTGTG GGTACTAGGT AAACCTTTAA	6215 6275 6335 6395
25	TAGAATGTTA ATGTTGTAT TCATTATAAG AATTTTGGC TGTTACTTAT TTACAACAAT ATTCACTCT AATTAGACAT TTACTAAACT TTCTCTGAA AACAAATGCCC AAAAAAGAAC ATTAGAAGAC ACGTAAGCTC AGTTGGTCTC TGCCACTAAG ACCAGCCAAC AGAAGCTTGA TTTATTCAA ACTTGCATT TTAGCATATT TTATCTGGA AAATTCAATT GTGTTGGTTT	6455 6515 6575 6635
30	TTGTTTTG TTTGTATTGA ATAGACTCTC AGAAATCCAA TTGTTGAGTA AATCTCTGG GTTTCTAAC CTTCTTTAG AT GTT ACC CTG TGT GAG GAG GCA TTC TTC AGG Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg	6695 6747
	180	185
35	TTT GCT GTT CCT ACA AAG TTT ACG CCT AAC TGG CTT AGT GTC TTG GTA Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val	6795
	190	195
40	195	200
45	GAC AAT TTG CCT GGC ACC AAA GTA AAC GCA GAG AGT GTA GAG AGG ATA Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile	6843
	205	210
50	210	215
	220	225
	230	235
	TGG AAA CAT CAA AAC AAA GAC CAA GAT ATA GTC AAG AAG ATC ATC CAA G	6940

Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln  
 240 245 250

5  
 GTAATTACAT TCCAAAATAC GTCTTGTAC GATTTGTAG TATCATCTCT CTCTCTGAGT 7000  
 TGAACACAAG GCCTCCAGCC ACATTCTTGG TCAAACATTAC ATTTCCCTT TCTTGAATCT 7060  
 10 TAACCAGCTA AGGCTACTCT CGATGCATTA CTGCTAAAGC TACCACTCAG AATCTCTCAA 7120  
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 15 AGTCCAAACT GTAGAATTCA CGTTGTGTGT TATTACTTTACCGAATGTCT GTATTATTAA 7300  
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Asp Ile Asp Leu Cys	
5 255	
GAA AAC AGC GTG CAG CGG CAC ATT GGA CAT GCT AAC CTC ACC TTC GAG	9022
Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr Phe Glu	
10 260 265 270	
CAG CTT CGT AGC TTG ATG GAA AGC TTA CCG GGA AAG AAA GTG GGA GCA	9070
Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala	
15 275 280 285	
GAA GAC ATT GAA AAA ACA ATA AAG GCA TGC AAA CCC AGT GAC CAG ATC	9118
Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile	
20 290 295 300	
CTG AAG CTG CTC AGT TTG TGG CGA ATA AAA AAT GGC GAC CAA GAC ACC	9166
Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr	
25 305 310 315 320	
TTG AAG GGC CTA ATG CAC GCA CTA AAG CAC TCA AAG ACG TAC CAC TTT	9214
Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe	
30 325 330 335	
CCC AAA ACT GTC ACT CAG AGT CTA AAG AAG ACC ATC AGG TTC CTT CAC	9262
Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His	
35 340 345 350	
AGC TTC ACA ATG TAC AAA TTG TAT CAG AAG TTA TTT TTA GAA ATG ATA	9310
Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile	
40 355 360 365	
GGT AAC CAG GTC CAA TCA GTA AAA ATA AGC TGC TTA TAACTGGAAA	9356
Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu	
45 370 375 380	
50	

5	TGGCCATTGA GCTGTTTCCT CACAATTGGC GAGATCCCAT GGATGAGTAA ACTGTTTCTC	9416
	AGGCACCTGA GGCTTTCAGT GATATCTTC TCATTACCAAG TGACTAATTT TGCCACAGGG	9476
	TACTAAAAGA AACTATGATG TGGAGAAAGG ACTAACATCT CCTCCAATAA ACCCAAATG	9536
	GTTAATCCAA CTGTCAGATC TGGATCGTTA TCTACTGACT ATATTTCCC TTATTACTGC	9596
10	TTGCAGTAAT TCAACTGGAA ATTAAAAAAA AAAAACTAGA CTCCACTGGG CCTTACTAAA	9656
	TATGGGAATG TCTAACTTAA ATAGCTTGG GATTCCAGCT ATGCTAGAGG CTTTATTAG	9716
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	TATTTATATT CATTAGATA TAAGATTGG ACATATTATC ATCCTATAA GAAACGGTAT	9836
15	GACTTAATT TAGAAAGAAA ATTATATTCT GTTTATTATG ACAAATGAAA GAGAAAATAT	9896
	ATATTTTAA TGAAAGTTT GTAGCATTAA TCTAATAGT ACTGCCATAT TTTCTGTGT	9956
	GGAGTATTT TATAATTAA TCTGTATAAG CTGTAATATC ATTTTATAGA AAATGCATTA	10016
20	TTTAGTCAAT TGTTTAATGT TGAAACAT ATGAAATATA AATTATCTGA ATATTAGATG	10076
	CTCTGAGAAA TTGAATGTAC CTTATTAAA AGATTTATG GTTTATAAC TATATAATG	10136
	ACATTATTAA AGTTTCAAA TTATTTTA TTGCTTCTC TGTTGCTTT ATT	10190

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### Claims

- 30 1. A protein characterized by the following properties:
  - (a) molecular weights on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) ; approximately 60 kD under reducing conditions ; approximately 60 kD and 120 kD under non-reducing conditions
  - (b) a high affinity to cation-exchange column and heparin column
  - (c) a biological activity to inhibit osteoclast differentiation and/or maturation
- 40 ; its activity is decreased by heating at 70°C for 10 min or at 56°C for 30 min. ; its activity is lost by heating at 90 °C for 10 min
- 45 2. A protein of claim 1 having N-terminal amino acid sequences provided in sequence number 7.
3. A protein of claim 1 produced in human fibroblasts.
- 50 4. A method of producing the protein of claim 1, 2, and 3 by the following process: cultivating human fibroblasts ; purifying the protein by a combination of ion-exchange column, affinity-column and reverse phase-column chromatography.
5. A method of producing the protein of claim 4 by cultivating human fibroblasts on alumina ceramic pieces.
- 55 6. A protein with amino acid sequence provided in sequence number 4.
7. cDNAs encoding amino acid sequence provided in sequence number 4.

8. cDNA with nucleotide sequence provided in sequence number 6.
9. cDNAs that hybridize to cDNA provided in sequence number 6 under moderately stringent conditions.
- 5 10. A protein expressed from cDNA encoding amino acid sequence provided in sequence number 4.
11. A protein with a biological activity to inhibit osteoclast differentiation and/or maturation, that obtain as amino acid expressed cDNA sharing at least 80 % sequence identity with the amino acid sequence provided in sequence number 4.
- 10 12. A method of production of the protein with the following properties and inhibit osteoclast differentiation and/or maturation by gene engineering using cDNA encoding amino acid sequence provided in sequence number 4:
  - (a) molecular weights on SDS-polyacrylamide gel electrophoresis (SDS-PAGE)
    - 15 ; approximately 60 kD under reducing conditions
    - ; approximately 60 kD and 120 kD under non-reducing conditions
  - (b) a high affinity to cation-exchange column and heparin column
  - 20 (c) ; inhibit osteoclast differentiation and/or maturation activity is decreased by heating at 70°C for 10 min or at 56°C for 30 min
    - ; its activity is lost by heating at 90 °C for 10 min
  - (d) internal amino acid sequence provided in sequence number 1-3.
13. A method of producing the protein according to claim 10 by gene engineering using mammalian cells as host cells.
14. A method of producing the protein according to claim 13 by gene engineering using 293/EBNA cells or CHO cells 30 as mammalian host cells.
15. A cDNA with nucleotide sequence provided in sequence number 8.
16. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 8.
- 35 17. cDNAs encoding amino acid sequence provided in sequence number 9.
18. A cDNA with nucleotide sequence provided in sequence number 10.
- 40 19. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 10.
20. cDNAs encoding amino acid sequence provided in sequence number 11.
21. A cDNA with nucleotide sequence provided in sequence number 12.
- 45 22. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 12.
23. cDNAs encoding amino acid sequence provided in sequence number 13.
- 50 24. A cDNA with nucleotide sequence provided in sequence number 14.
25. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 14.
26. cDNAs encoding amino acid sequence provided in sequence number 15.
- 55 27. A cDNA with nucleotide sequence provided in sequence number 83.
28. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 83.

29. cDNAs encoding amino acid sequence provided in sequence number 62.

30. A cDNA with nucleotide sequence provided in sequence number 84.

5 31. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 84.

32. cDNAs encoding amino acid sequence provided in sequence number 63.

33. A cDNA with nucleotide sequence provided in sequence number 85.

10 34. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 85.

35. cDNAs encoding amino acid sequence provided in sequence number 64.

15 36. A cDNA with nucleotide sequence provided in sequence number 86.

37. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 86.

38. cDNAs encoding amino acid sequence provided in sequence number 65.

20 39. A cDNA with nucleotide sequence provided in sequence number 87.

40. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 87.

25 41. cDNAs encoding amino acid sequence provided in sequence number 66.

42. A cDNA with nucleotide sequence provided in sequence number 88.

43. A protein encoded by a cDNA having a sequence provided in sequence number 88.

30 44. cDNAs encoding amino acid sequence provided in sequence number 67.

45. A cDNA with nucleotide sequence provided in sequence number 89.

35 46. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 89.

47. cDNAs encoding amino acid sequence provided in sequence number 68.

48. A cDNA with nucleotide sequence provided in sequence number 90.

40 49. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 90.

50 50. cDNAs encoding amino acid sequence provided in sequence number 69.

45 51. A cDNA with nucleotide sequence provided in sequence number 91.

52. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 91.

53. cDNAs encoding amino acid sequence provided in sequence number 70.

50 54. A cDNA with nucleotide sequence provided in sequence number 92.

55. A protein encoded by a cDNA having a nucleotide sequence provided in number 92.

55 56. cDNAs encoding amino acid sequence provided in sequence number 71.

57. A cDNA with nucleotide sequence provided in sequence number 93.

58. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 93.

59. cDNAs encoding amino acid sequence provided in sequence number 72.

5 60. A cDNA with nucleotide sequence provided in sequence number 94.

61. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 94.

62. cDNAs encoding amino acid sequence provided in sequence number 73.

10 63. A cDNA with nucleotide sequence provided in sequence number 95.

64. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 95.

15 65. cDNAs encoding amino acid sequence provided in sequence number 74.

66. A cDNA with nucleotide sequence provided in sequence number 96.

67. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 96.

20 68. cDNAs encoding amino acid sequence provided in sequence number 75.

69. A cDNA with nucleotide sequence provided in sequence number 97.

25 70. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 97.

71. cDNAs encoding amino acid sequence provided in sequence number 76.

72. A cDNA with nucleotide sequence provided in sequence number 98.

30 73. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 98.

74. cDNAs encoding amino acid sequence provided in sequence number 77.

35 75. A cDNA with nucleotide sequence provided in sequence number 99.

76. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 99.

77. cDNAs encoding amino acid sequence provided in sequence number 78.

40 78. A cDNA with nucleotide sequence provided in sequence number 100.

79. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 100.

45 80. cDNAs encoding amino acid sequence provided in sequence number 79.

81. A cDNA with nucleotide sequence provided in sequence number 101.

82. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 101.

50 83. cDNAs encoding amino acid sequence provided in sequence number 80.

84. A cDNA with nucleotide sequence provided in sequence number 102.

55 85. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 102.

86. cDNAs encoding amino acid sequence provided in sequence number 81.

87. A cDNA with nucleotide sequence provided in sequence number 103.

88. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 103.

5 89. cDNAs encoding amino acid sequence provided in sequence number 82.

90. Genomic DNAs encoding the amino acid sequence provided in sequence number 4.

91. Genomic DNAs of Claim 90 with the nucleotide sequence provided in sequence number 104 or 105.

10 92. An antibody having specific affinity to the OCIF

93. An antibody of Claim 92 that is polyclonal antibody.

15 94. An antibody of Claim 92 that is monoclonal antibody.

95. A monoclonal antibody of Claim 94 being characterized by the following properties.  
Molecular weight of about 150,000, and of subclass IgG<sub>1</sub>, IgG<sub>2a</sub>, or IgG<sub>2b</sub>.

20 96. A method of determining the concentration of the protein of the OCIF using the antibodies of Claim 92, 93, 94, and 95.

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Fig. 1

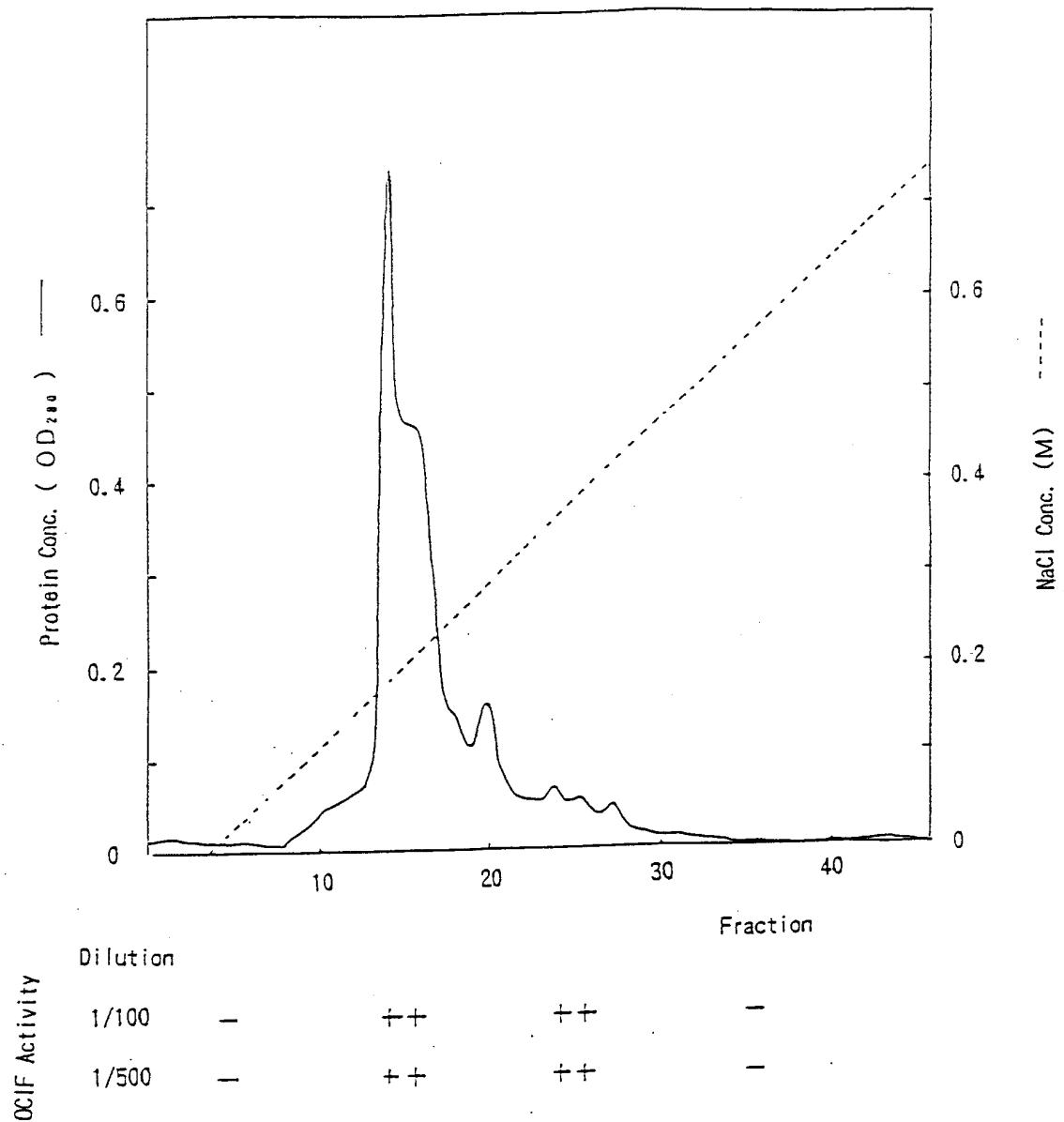


Fig. 2

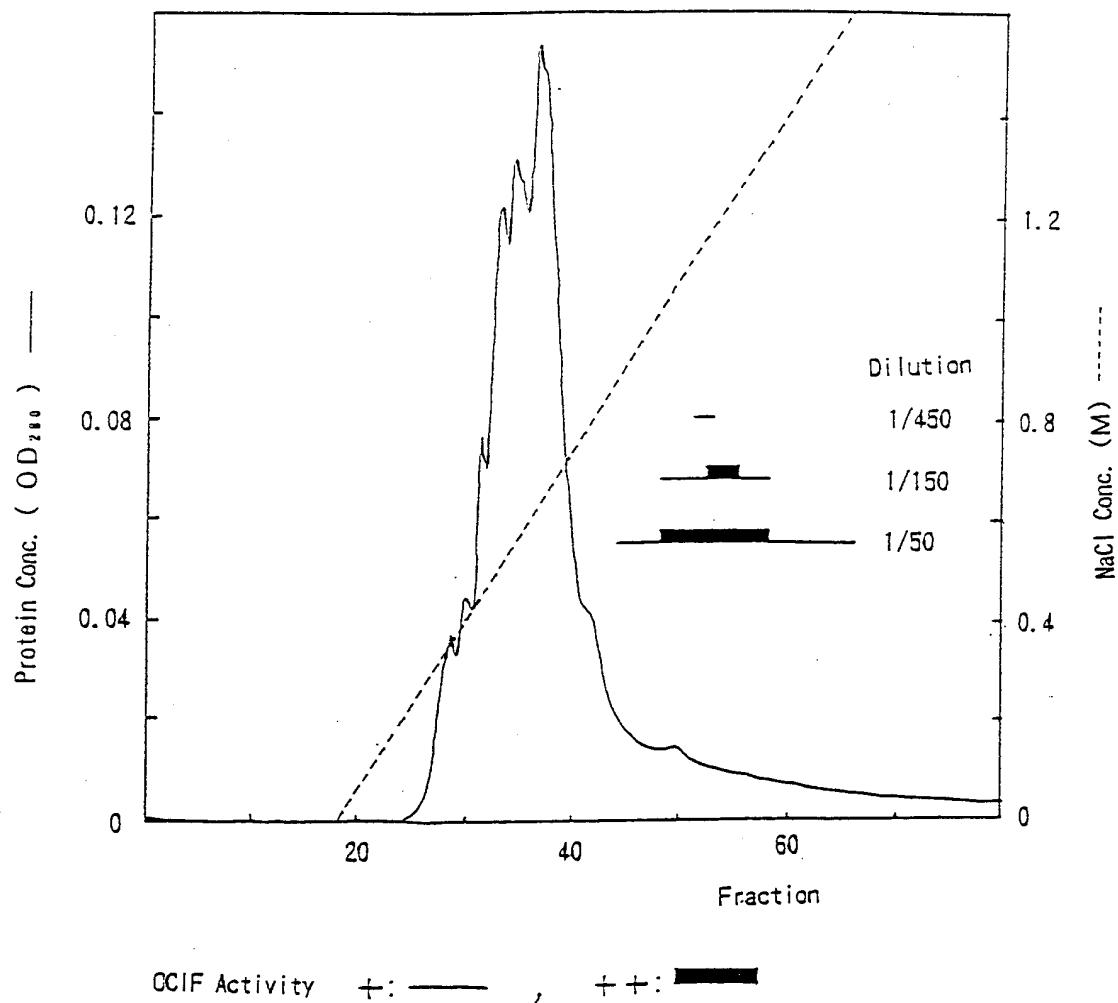


Fig. 3

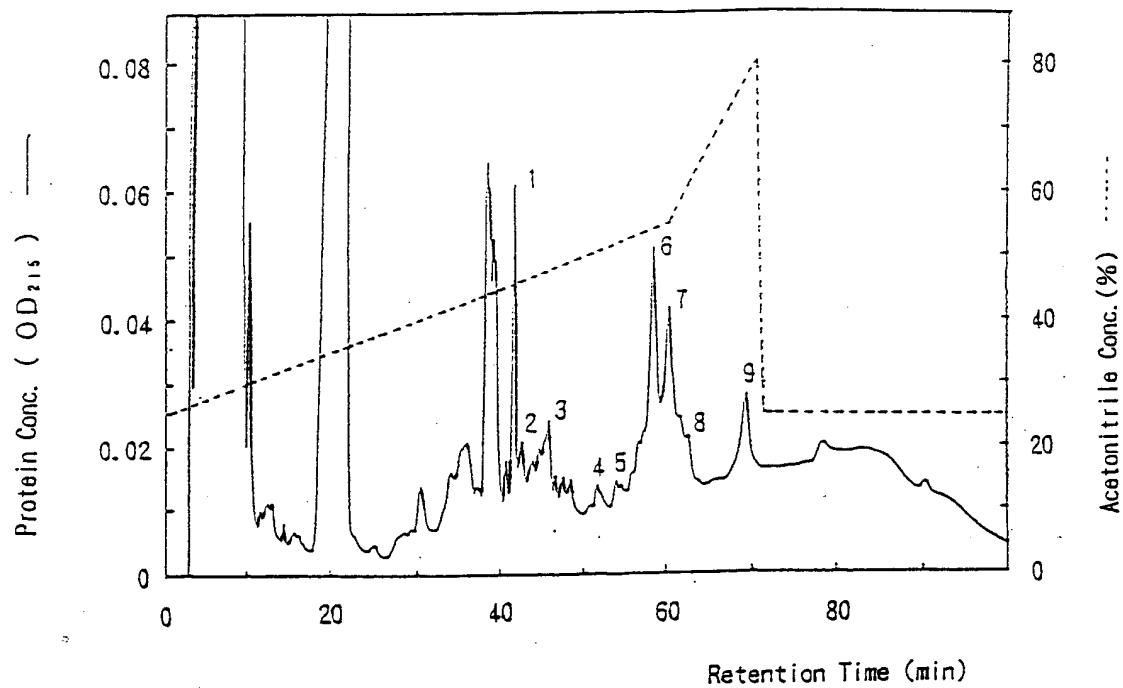


Fig. 4

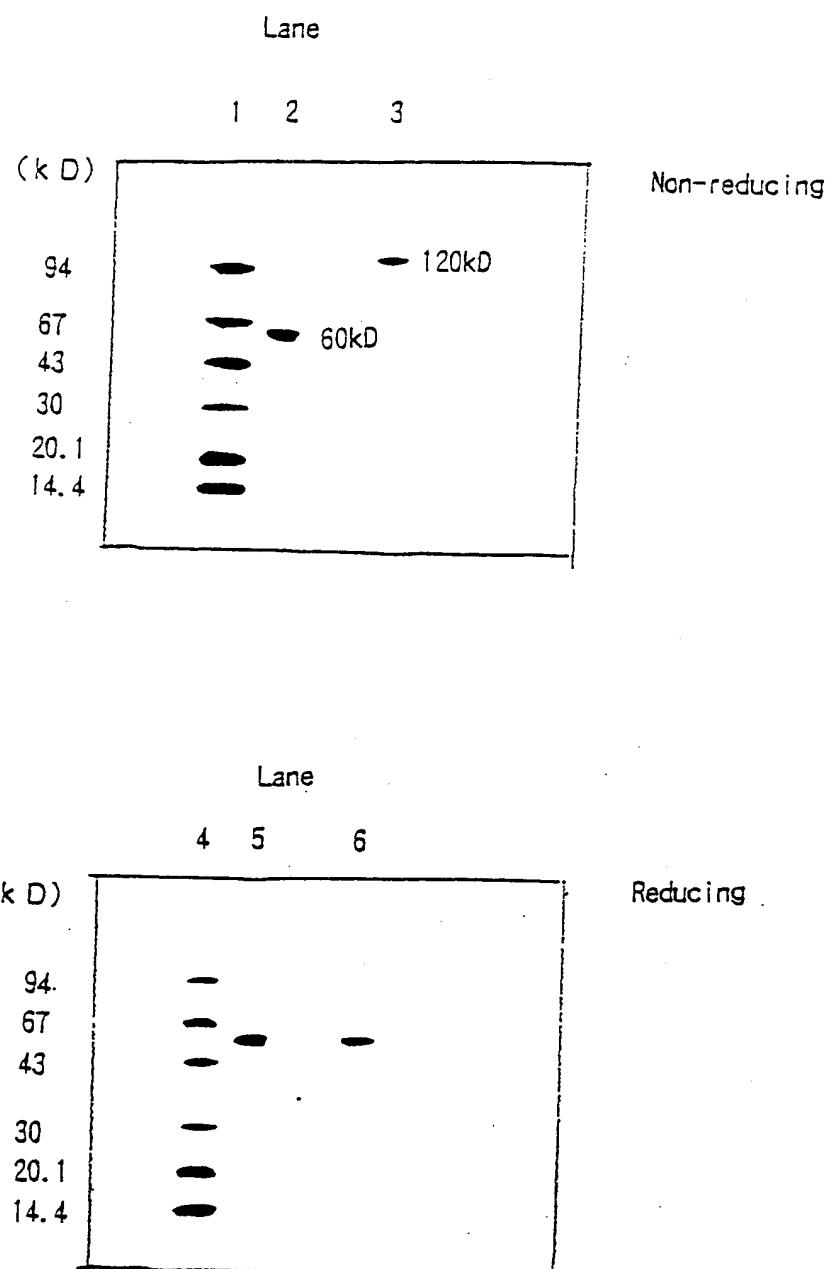
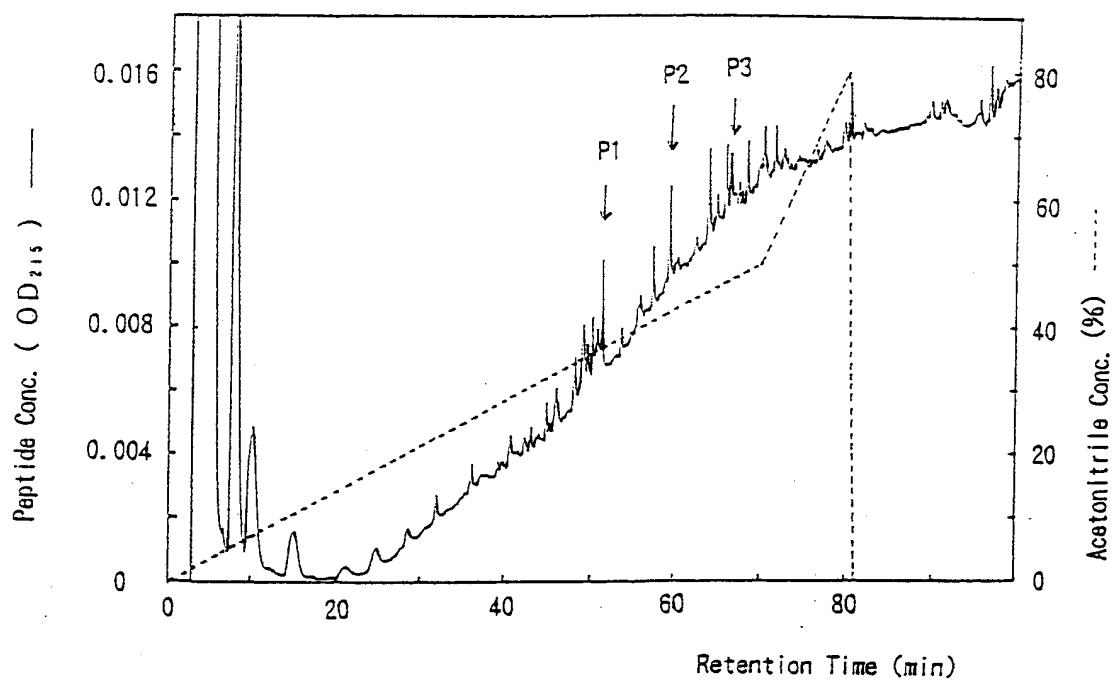
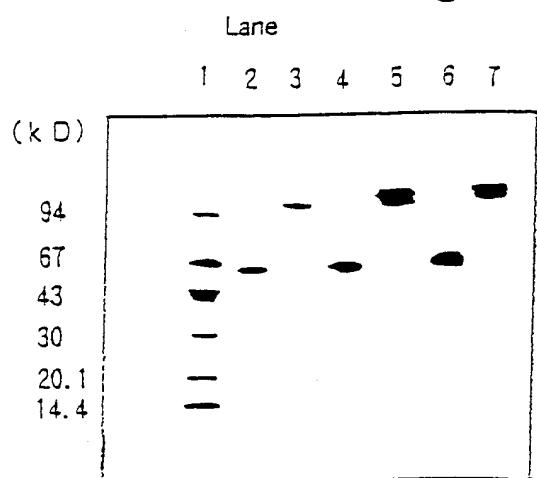


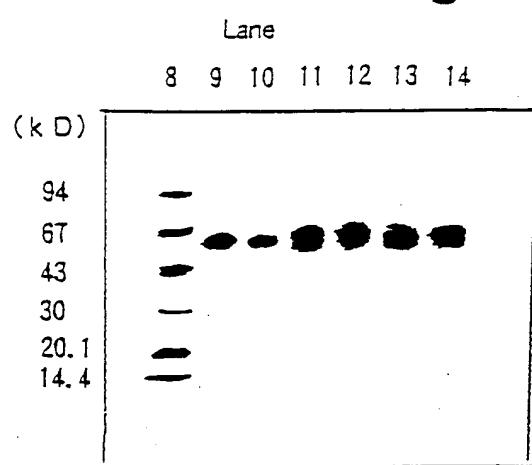
Fig.5



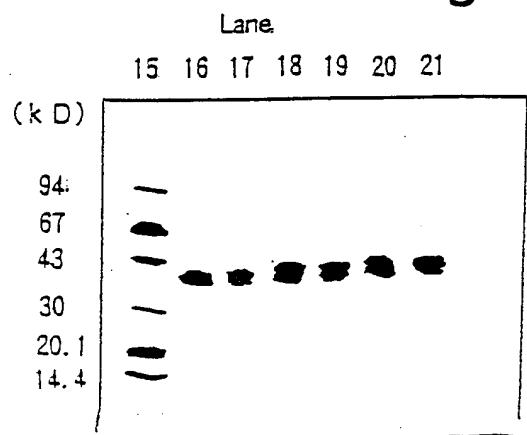
**Fig. 6**



**Fig. 7**



**Fig. 8**



## Fig. 9

1  
 MNNLLCCALVFLDISIKWTTQETFPPKYLHYDEETSHQLLCOKCPPGTYLKQHCTAKWKT (OCIF1)  
 \*\*\*\*\*  
 MNNLLCCALVFLDISIKWTTQETFPPKYLHYDEETSHQLLCOKCPPGTYLKQHCTAKWKT (OCIF2)  
 1

61  
 VCAPCPDHYYTDSWHTSDECLYCSPECVELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF1)  
 \*\*\*\*\*  
 VCAPCPDHYYTDSWHTSDECLYCSPEC-----CNRTHNRVCECKEGRYLEIEFCLK (OCIF2)  
 61

121  
 HRSCPPGFGVVQAGTPERNTVCKRCPDGFFSNETSSKAPCRKHTNCSVFGLLLQKGNAT (OCIF1)  
 \*\*\*\*\*  
 HRSCPPGFGVVQAGTPERNTVCKRCPDGFFSNETSSKAPCRKHTNCSVFGLLLQKGNAT (OCIF2)  
 114

181  
 HDNICSGNSESTQKCGIDVTLCEEAFFRFAVPTKFTPWNLSVLVDNLPGTKVNAESVERI (OCIF1)  
 \*\*\*\*\*  
 HDNICSGNSESTQKCGIDVTLCEEAFFRFAVPTKFTPWNLSVLVDNLPGTKVNAESVERI (OCIF2)  
 174

241  
 KRQHSSQEQTFLKLWKHQNKDQDIVKKIIQDIDLCENSVQRHIGHANLTFEQLRSLME (OCIF1)  
 \*\*\*\*\*  
 KRQHSSQEQTFLKLWKHQNKDQDIVKKIIQDIDLCENSVQRHIGHANLTFEQLRSLME (OCIF2)  
 234

301  
 SLPGKKVGAEDIEKTAKPSDQILKLLSLWRIKNGDQDTLKGLMHALKHSKTYHFPKT (OCIF1)  
 \*\*\*\*\*  
 SLPGKKVGAEDIEKTAKPSDQILKLLSLWRIKNGDQDTLKGLMHALKHSKTYHFPKT (OCIF2)  
 294

361  
 VTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL (OCIF1)  
 \*\*\*\*\*  
 VTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL (OCIF2)  
 354

## Fig. 10

1  
 MNNLLCCALVFLDISIKWTTQETFPPKYLHYDEETSHQLLCOKCPPGTYLKQHCTAKWKT (OCIF1)  
 \*\*\*\*\*  
 MNKLLCCALVFLDISIKWTTQETFPPKYLHYDEETSHQLLCOKCPPGTYLKQHCTAKWKT (OCIF3)  
 1

61  
 VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF1)  
 \*\*\*\*\*  
 VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF3)  
 61

121  
 HRSCPPGFGVVQAGTPERNTVCKRCPDGFFSNETSSKAPCRKHTNCVFGLLLQKGNAT (OCIF1)  
 \*\*\*\*\*  
 HRSCPPGFGVVQAGTPERNTVCKRCPDGFFSNETSSKAPCRKHTNCVFGLLLQKGNAT (OCIF3)  
 121

181  
 HDNICSGNSESTQKCGIDVTLCEEAFFRFAVPTKFTPWNLSVLVDNLPGTKVNAESVERI (OCIF1)  
 \*\*\*\*\*  
 HDNICSGNSESTQKCGIDVTLCEEAFFRFAVPTKFTPWNLSVLVDNLPGTKVNAESVERI (OCIF3)  
 181

241  
 KRQHSSQEQTQFQLLKLWKHQNKDQDIVKKIIQDIDLCENSQQRHIGHANLTFEQLRSLME (OCIF1)  
 \*\*\*\*\*  
 KRQHSSQEQTQFQLLKLWKHQNKDQDIVKKIIQDIDLCENSQQRHIGHANLS----- (OCIF3)  
 241

301  
 SLPGKKVGAEDIEKTIKACKPSDQILKLLSLWRIKNGDQDTLKGLMHALKHSKTYHFPKT (OCIF1)  
 \*\*\*\*\*  
 -----LWRIKNGDQDTLKGLMHALKHSKTYHFPKT (OCIF3)  
 292

361  
 VTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL (OCIF1)  
 \*\*\*\*\*  
 VTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL (OCIF3)  
 322

**Fig. 11**

1  
MNNLLCCALVFLDISIKWTTQETFPPKYLHYDEETSHQLLCOKCPPGTYLKQHCTAKWKT (OCIF1)  
\*\*\*\*\*  
MNKLLCCSLVFLDISIKWTTQETFPPKYLHYDEETSHQLLCOKCPPGTYLKQHCTAKWKT (OCIF4)  
1  
  
61  
VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF1)  
\*\*\*\*\*  
VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF4)  
61  
  
121  
HRSCPPGFGVVQAGTPERNTVCKRCPDGFFSNETSSKAPCRKHTNCSVFGLLLTKGNAT (OCIF1)  
\*\*\*\*\*  
HRSCPPGFGVVQAGTCQCAAKLIRIMQSQIVVTV (OCIF4)  
121

**Fig. 12**

1  
MNNLLCCALVFLDISIKWTTQETFPPKYLHYDEETSHQLLCOKCPPGTYLKQHCTAKWKT (OCIF1)  
\*\*\*\*\*  
MNKLLCCALVFLDISIKWTTQETFPPKYLHYDEETSHQLLCOKCPPGTYLKQHCTAKWKT (OCIF5)  
1  
  
61  
VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF1)  
\*\*\*\*\*  
VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF5)  
61  
  
121  
HRSCPPGFGVVQAGTPERNTVCKRCPDGFFSNETSSKAPCRKHTNCSVFGLLLTKGNAT (OCIF1)  
\*\*\*\*\* \*  
HRSCPPGFGVVQAGCRRRPKPQICI (OCIF5)  
121

Fig. 13

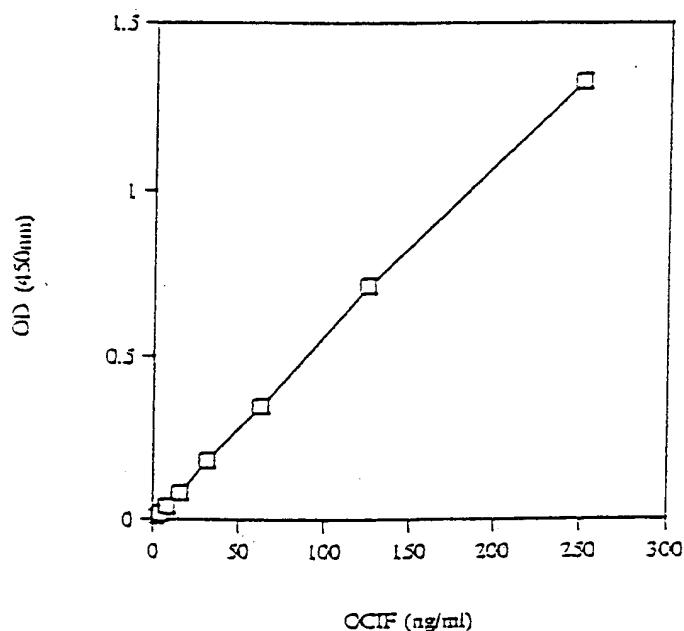


Fig. 14

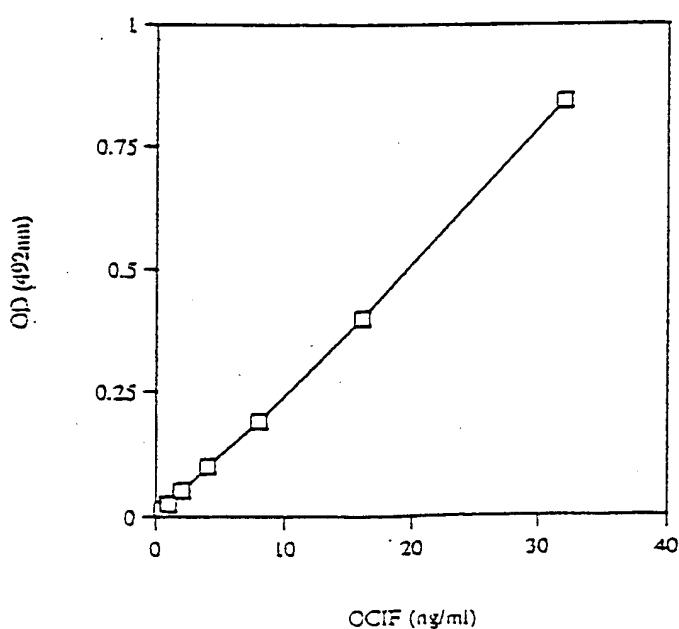
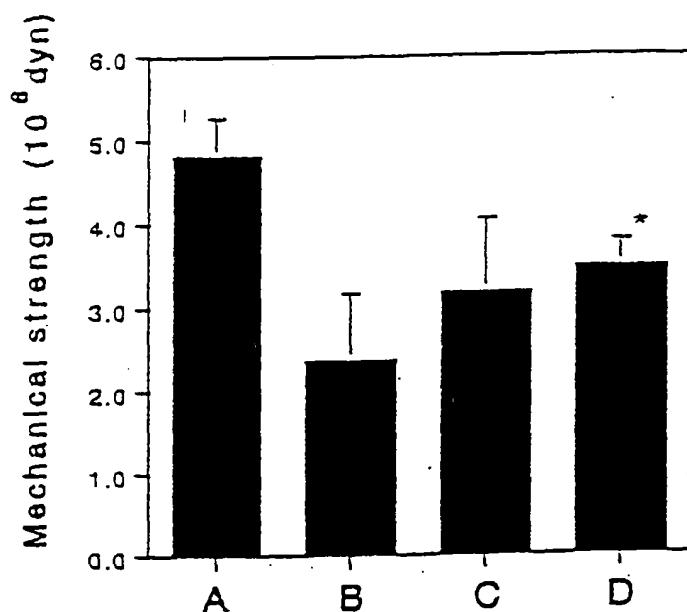


Fig. 15



- A : Normal rat
- B : Denerved rat + Vehicle
- C : Denerved rat + OCIF 10 $\mu$ g/kg/day
- C : Denerved rat + OCIF 100 $\mu$ g/kg/day

INTERNATIONAL SEARCH REPORT		International application No. PCT/JP96/00374									
<b>A. CLASSIFICATION OF SUBJECT MATTER</b> Int. Cl <sup>6</sup> C07K14/52, C07K16/24, C12N15/19, C12N15/06, C12N5/08, C12N5/10, C12N5/20, C12P21/02, C12P21/08, G01N33/577 According to International Patent Classification (IPC) or to both national classification and IPC											
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) Int. Cl <sup>6</sup> C07K14/52, C07K16/24, C12N15/19, C12N15/06, C12N5/08, C12N5/10, C12N5/20, C12P21/02, C12P21/08, G01N33/577											
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched											
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) BIOSIS PREVIEWS, CAS ONLINE, WPI, WPI/L											
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: left; padding: 2px;">Category*</th> <th style="text-align: left; padding: 2px;">Citation of document, with indication, where appropriate, of the relevant passages</th> <th style="text-align: left; padding: 2px;">Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td style="padding: 2px;">A</td> <td style="padding: 2px;">Fawthrop, F.W. et al. "The effect of transforming growth factor beta on the plasminogen activator activity of normal human osteoblast-like cells and a human osteosarcoma cell line MG-63", J. Bone. Miner. Res. (1992) Vol. 7, No. 12, p. 1363-1371</td> <td style="padding: 2px;">1 - 96</td> </tr> <tr> <td style="padding: 2px;">A</td> <td style="padding: 2px;">Fenton, A.J. et al. "Long-term culture of disaggregated rat osteoclasts inhibition of bone resorption and reduction of osteoclast-like cell number by calcitonin and PTHrP107-139", J. Cell Physiol. (1993) Vol. 155, No. 1, p. 1-7</td> <td style="padding: 2px;">1 - 96</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	A	Fawthrop, F.W. et al. "The effect of transforming growth factor beta on the plasminogen activator activity of normal human osteoblast-like cells and a human osteosarcoma cell line MG-63", J. Bone. Miner. Res. (1992) Vol. 7, No. 12, p. 1363-1371	1 - 96	A	Fenton, A.J. et al. "Long-term culture of disaggregated rat osteoclasts inhibition of bone resorption and reduction of osteoclast-like cell number by calcitonin and PTHrP107-139", J. Cell Physiol. (1993) Vol. 155, No. 1, p. 1-7	1 - 96
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Date of the actual completion of the international search May 14, 1996 (14. 05. 96)		Date of mailing of the international search report May 28, 1996 (28. 05. 96)									
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.		Authorized officer Telephone No.									